(54) Title: SIMULTANEOUS DETECTION, IDENTIFICATION AND DIFFERENTIATION OF EUBACTERIAL TAXA USING A HYBRIDIZATION ASSAY

(57) Abstract

The present invention relates to a method for detection and identification of at least one microorganism, or for the simultaneous detection of several microorganisms in a sample, comprising the steps of: (i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample; (ii) if need be amplifying the 16S-23S rRNA spacer region, or a part of it, with at least one suitable primer pair; (iii) hybridizing the polynucleic acids of step (i) or (ii) with at least one and preferably more than one of the spacer probes as mentioned in table 1a or equivalents of thereof, under the appropriate hybridization and wash conditions, and/or with a taxon-specific probe derived from any of the spacer sequences as represented in figs. 1-103 under the same hybridization and wash conditions; (iv) detecting the hybrids formed in step (iii) with each of the probes used under appropriate hybridization and wash conditions; (v) identification of the microorganism(s) present in the sample from the differential hybridization signals obtained in step (iv).

SIMULTANEOUS DETECTION, IDENTIFICATION AND DIFFERENTIATION OF EUBACTERIAL TAXA USING A HYBRIDIZATION ASSAY

The present invention relates to nucleic acid probes derived from the spacer region between the 16S and 23S ribosomal ribonucleic acid (rRNA) genes, to be used for the

5   specific detection of eubacterial organisms in a biological sample by a hybridization procedure, as well as to nucleic acid primers to be used for the amplification of said spacer region of eubacterial organisms in a biological sample. The present invention also relates to new spacer region sequences from which said probes or primers may be derived.

Since the advent of the polymerase chain reaction and some other nucleic acid

10   amplification techniques the impact of DNA-probe technology in the diagnosis of micro-organisms in biological samples of all sorts is increasing. Being often more specific and potentially more sensitive - if an adequate amplification and/or detection system is used -the DNA probe approach may eventually replace the conventional identification techniques.

The reliability of nucleic acid based tests essentially depends on the sensitivity and

15   specificaty of the probes and/or primers used. Thus the corner stone of this type of assay is the identification of nucleic acid sequences which are unique to the group of organisms of interest.

Most of the nucleic acid based tests either described in literature and/or commercially available aim at the detection of just one particular organism in a biological sample. Since

20   most biological samples usually may contain a great variety of clinically relevant micro-organisms, a multitude of separate assays have to be performed to detect all relevant organisms possibly present. This approach would be very expensive, laborious and time-consuming. Consequently, the number of tests actually performed in most routine diagnostic labs on a particular sample is restricted to the detection of just a few of the most relevant

25   organisms. Therefore it would be extremely convenient to have access to a system which enables the fast, easy and simultaneous detection of a multitude of different organisms. The more organisms that can be screened for in the same assay, the more cost-effective the procedure would be.

As put forward in earlier published documents, the spacer region situated between the

30   16S rRNA and the 23S rRNA gene, also referred to as the internal transcribed spacer (ITS), is an advantageous target region for probe development for detection of pathogens of

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bacterial origin (International application WO 91/16454; Rossau et al., 1992; EP-A-0 395 292).

One of its most appreciated advantages is that sequences unique to a great variety of bacterial taxa can be found in a very limited area of the bacterial genome. This characteristic allows for an advantageous design of "probe-panels" enabling the simultaneous detection of a set of organisms possibly present in a particular type of a biological sample. Moreover, being flanked by quasi-universally conserved nucleotide sequences - more particularly located in the 3'-part of the 16S rRNA gene and the 5'-part of the 23S rRNA gene respectively - almost all spacers can be simultaneously amplified with a limited set of amplification primers. Alternatively, specific primer sets can be derived from the spacer sequences themselves, thereby allowing species- or group-specific amplifications.

The 16S-23S rRNA spacer region is a relatively short (about 200 to 1000 base pairs) stretch of DNA present in one or multiple copies in the genome of almost all eubacterial organisms. If multiple copies are present in the genome of one bacterium these copies can either be identical (as is most probably the case in some Neisseria species) or may differ from each other (as is the case for E. coli). This difference can be limited to a few nucleotides but also deletions and insertions of considerable length may be present.

Uptil now, spacer probes are only described and made publicly available for a limited number of organisms many of which were disclosed in international application WO 91/16454. As described above, it would be very advantageous to be able to detect simultaneously a panel of pathogens: e.g. a panel of pathogens possibly present in the same type of biological sample, or a panel of pathogens possibly causing the same type of disease symptoms, which are difficult to differentiate clinically and/or biochemically, or a panel of organisms belonging to the same taxon. In order to make the different panels as complete as possible, additional probes or sets of probes located in the spacer region and enabling the identification of at least the following bacterial groups or species are required :

- Mycobacterium species
- Listeria species
- Chlamydia species
- Acinetobacter species
- Mycoplasma species
- Streptococcus species

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- <u>Staphylococcus</u> species
- <u>Salmonella</u> species
- <u>Brucella</u> species
- <u>Yersinia</u> species
5 - <u>Pseudomonas</u> species

These additional spacer probes need to be meticulously designed such that they can be used simultaneously with at least one other probe, under the same hybridization and wash conditions, allowing the detection of a particular panel of organisms.

It is thus the aim of the present invention to select probes or sets of probes, which 10 have as target the 16S-23S rRNA spacer region, and which allow the detection and identification of at least one, and preferably more than one, of the above mentioned micro-organisms. The probes or probe sets are selected in such a way that they can be used in combination with at least one other probe, preferably also originating from the 16S-23S rRNA spacer region, under the same hybridisation and wash conditions, to allow possibly 15 the simultaneous detection of several micro-organisms in a sample.

It is also an aim of the present invention to provide for a selection method for use in the selection of said spacer probes or probe sets.

It is also an aim of the present invention to provide a rapid and reliable hybridization method for detection and identification of at least one micro-organism in a sample, or for the 20 simultaneous detection and identification of several micro-organisms in a sample.

It is more particularly an aim of the present invention to provide a hybridization method allowing simultaneous detection and identification of a set of micro-organisms, liable to be present in a particular type of sample.

It is more particularly an aim of the present invention to provide probes or sets of 25 probes for the possible simultaneous detection of micro-organisms in a sample originating from respiratory tract.

It is another particular aim of the present invention to provide probes or sets of probes for the possible simultaneous detection of micro-organisms in a sample originating from cerebrospinal fluid.

30 It is still another particular aim of the present invention to provide probes or sets of probes for the possible simultaneous detection of micro-organisms in a sample originating from urogenital tract.

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It is still another particular aim of the present invention to provide probes or sets of probes for the possible simultaneous detection of micro-organisms in a sample taken from the gastro-intestinal tract of a patient.

It is still another particular aim of the present invention to provide probes or sets of
5   probes for the possible simultaneous detection of micro-organisms in a sample originating from food or environmental samples.

It is moreover an aim of the present invention to provide a method for detection and identification of a particular taxon in a sample, or a set of particular taxa, said taxon being either a complete genus, or a subgroup within a genus, a species or even subtypes within a
10   species (subspecies, serovars, sequevars, biovars...).

It is more particularly an aim of the present invention to provide probes or sets of probes for the detection of <u>Mycobacterium</u> species and subspecies, more particularly for the detection of <u>M</u>. <u>tuberculosis</u> complex strains, <u>Mycobacterium</u> strains from the MAIS-complex, <u>M</u>. <u>avium</u> and <u>M</u>. <u>paratuberculosis</u>, <u>M</u>. <u>intracellulare</u> and <u>M</u>. <u>intracellulare</u>-like
15   strains, <u>M</u>. <u>scrofulaceum</u>, <u>M</u>. <u>kansasii</u>, <u>M</u>. <u>chelonae</u>, <u>M</u>. <u>gordonae</u>, <u>M</u>. <u>ulcerans</u>, <u>M</u>. <u>genavense</u>, <u>M</u>. <u>xenopi</u>, <u>M</u>. <u>simiae</u>, <u>M</u>. <u>fortuitum</u>, <u>M</u>. <u>malmoense</u>, <u>M</u>. <u>celatum</u> and <u>M</u>. <u>haemophilum</u>.

It is also an aim of the present invention to provide probes or sets of probes for the detection of <u>Mycoplasma</u> strains, more particularly of <u>M</u>. <u>pneumoniae</u> and <u>M</u>. <u>genitalium</u>.
20   It is also an aim of the present invention to provide probes or sets of probes for the detection of <u>Pseudomonas</u> strains, more particularly <u>P</u>. <u>aeruginosa</u>.

It is also an aim of the present invention to provide probes or sets of probes for detection of <u>Staphylococcus</u> species, more particularly <u>S</u>. <u>aureus</u> and <u>S</u>. <u>epidermidis</u>.

It is also an aim of the present invention to provide probes or sets of probes for the
25   detection of <u>Acinetobacter</u> strains, more particularly <u>A</u>. <u>baumanii</u>.

It is also an aim of the present invention to provide probes or sets of probes for the detection of <u>Listeria</u> strains, more particularly <u>Listeria</u> <u>monocytogenes</u>.

It is also an aim of the present invention to provide probes or sets of probes for the detection of <u>Brucella</u> strains.
30   It is also an aim of the present invention to provide probes or sets of probes for the detection of <u>Salmonella</u> strains.

It is also an aim of the present invention to provide probes or sets of probes for the

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detection of <u>Chlamydia</u> strains, more particularly <u>C</u>. <u>trachomatis</u> and <u>C</u>. <u>psittaci</u>.

It is also an aim of the present invention to provide probes or sets of probes for the detection of <u>Streptococcus</u> strains.

It is also an aim of the present invention to provide probes or sets of probes for the
5   detection of <u>Yersinia enterolitica</u> strains.

It is also an aim of the present invention to provide primers allowing specific amplification of the 16S-23S rRNA spacer region for certain organisms. More particularly, it is an aim of the present invention to provide primers for the specific amplification of the spacer region of <u>Mycobacterium</u>, <u>Chlamydia</u>, <u>Listeria</u>, <u>Brucella</u> and <u>Yersinia enterolitica</u>
10   strains.

It is also an aim of the present invention to provide new sequences of 16S-23S rRNA spacer regions from which useful spacer probes or primers can be derived.

It is also an aim of the present invention to provide for kits for detection of at least one organism in a sample in which said probes and/or primers are used.

15   It is noted that for a few of the above-mentioned organisms spacer sequences have already been published in literature or in publicly accessable data-banks.

However, it should be made clear that the spacer region sequences disclosed in the current invention (figs. 1-103) are new and, in case they originate from the same species as those of which a spacer sequence was already described in the prior art, they differ to some
20   extent from the already described sequences.

Moreover, it is the principal aim of the present invention to select, from the compilation of sequence data on spacer regions, specific probes and sets of probes enabling the detection and identification of a particular panel of organisms, be it the organisms belonging to a common taxon, or the organisms possibly present in the same type of sample.

25   The selection procedure usually consists of a theoretical and an experimental part. First of all, the different spacer sequences need to be aligned to those of the 'closest neighbours' or to the spacer sequences of other micro-organisms liable to be present in the same sample. This requires of course the sequence determination of the spacer region, as described in the examples. From the alignment, regions of divergence can be defined, from
30   which probes with desired hybridization characteristics are designed, according to guidelines known to the man skilled in the art and specified in more detail below.

Secondly, the designed probes need to be tested experimentally and evaluated for their

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usefulness under specific hybridization conditions and/or in combination with other probes. Experimental testing can be done according to any hybridization method known in the art, but a preferred assay for the simultaneous testing of different probes under the same conditions is the reverse hybridization assay. A specific format for reverse hybridization of different probes simultaneously used in the current invention is the LiPA (Line Probe Assay) as described below.

Upon experimental testing unexpected hybridization behaviour may show up when the probes are hybridized to the target nucleic acid, and specific probe adaptations may be required.

Moreover, specificity and sensitivity of the probes need to be tested with a large collection of strains, both belonging to the taxon to be detected and belonging to other taxa. Due to genome heterogeneity in the spacer region, or the existence of multiple spacer regions with different sequences in the same organism, it is quite often necessary to sequence spacer regions of additional strains, or to sequence additional spacer regions in the same strain, and redesign the probes according to the new sequence data in order to obtain a better sensitivity and/or specificity (see e.g. example 3). In some cases it may be necessary or preferable to use several probes for the same organism (see e.g. example 2 and 7). Also, upon sequencing the spacer region, some organisms may show unexpected (un)relatedness, which may lead to a revision of strain classification contrary to classical taxonomic criteria (see e.g. examples 2 and 7).

In conclusion, the experimental part of the probe selection procedure is indispensable and complementary to the theoretical part. Probe design, especially under the fixed conditions of reverse hybridization (the same conditions for each probe) is not straightforward and probes have to be evaluated meticulously before they can be used in a reverse hybridization format. Therefor, probes cannot always be simply derived on a theoretical basis from a known gene sequence.

For designing probes with desired characteristics the following useful guidelines may be followed.

Because the extent and specificity of hybridization reactions such as those described herein are affected by a number of factors, manipulation of one or more of those factors will determine the exact sensitivity and specificity of a particular probe, whether perfectly complementary to its target or not. The importance and effect of various assay conditions,

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explained further herein, are known to those skilled in the art.

First, the stability of the [probe : target] nucleic acid hybrid should be chosen to be compatible with the assay conditions. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs, and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %GC result in a Tm about 2-10°C higher than the temperature at which the final assay will be performed. The base composition of the probe is significant because G-C base pairs exhibit greater thermal stability as compared to A-T base pairs due to additional hydrogen bonding. Thus, hybridization involving complementary nucleic acids of higher G-C content will be stable at higher temperatures.

Conditions such as ionic strength and incubation temperature under which a probe will be used should also be taken into account in constructing a probe. It is known that hybridization will increase as the ionic strenght of the reaction mixture increases, and that the thermal stability of the hybrids will increase with increasing ionic strenght. On the other hand, chemical reagents, such as formamide, urea, DMSO and alcohols, which disrupt hydrogen bonds, will increase the stringency of hybridization. Destabilization of the hydrogen bonds by such reagents can greatly reduce the Tm. In general, optimal hybridization for synthetic oligonucleotide probes of about 10-50 bases in length occurs approximately 5°C below the melting temperature for a given duplex. Incubation at temperatures below the optimum may allow mismatched base sequences to hybridize and can therefore result in reduced specificity.

It is desirable to have probes which hybridize only under conditions of high stringency. Under high stringency conditions only highly complementary nucleic acid hybrids will form; hybrids without a sufficient degree of complementarity will not form. Accordingly, the stringency of the assay conditions determines the amount of complementarity needed between two nucleic acid strands forming a hybrid. Stringency is chosen to maximize the difference in stability between the hybrid formed with the target and the nontarget nucleic acid. In some examples of the current invention, e.g. when highly related organisms need to be differentiated, it may be necessary to detect single base pair changes. In those cases, conditions of very high stringency are needed.

Second, probes should be positioned so as to minimize the stability of the [probe : nontarget] nucleic acid hybrid. This may be accomplished by minimizing the length of perfect

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complementarity to non-target organisms, avoiding GC rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible. Whether a probe sequence is useful to detect only a specific type of organism depends largely on the thermal stability difference between [probe:target] hybrids and [probe:nontarget] hybrids. In designing probes, the differences in these Tm values should be as large as possible (e.g. at least 2°C and preferably 5°C).

The length of the target nucleic acid sequence and, accordingly, the length of the probe sequence can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired hybridization characteristics. In other cases, one sequence may be significantly better than another which differs merely by a single base. While it is possible for nucleic acids that are not perfectly complementary to hybridize, the longest stretch of perfectly complementary base sequence will normally primarily determine hybrid stability. While oligonucleotide probes of different lengths and base composition may be used, oligonucleotide probes preferred in this invention are between about 10 to 50 bases in length and are sufficiently homologous to the target nucleic acid.

Third, regions in the target DNA or RNA which are known to form strong internal structures inhibitory to hybridization are less preferred. Likewise, probes with extensive self-complementarity should be avoided. As explained above, hybridization is the association of two single strands of complementary nucleic acids to form a hydrogen bonded double strand. It is implicit that if one of the two strands is wholly or partially involved in a hybrid that it will be less able to participate in formation of a new hybrid. There can be intramolecular and intermolecular hybrids formed within the molecules of one type of probe if there is sufficient self complementarity. Such structures can be avoided through careful probe design. By designing a probe so that a substantial portion of the sequence of interest is single stranded, the rate and extent of hybridization may be greatly increased. Computer programs are available to search for this type of interaction. However, in certain instances, it may not be possible to avoid this type of interaction.

The probes of the present invention are designed for attaining optimal performance under the same hybridization conditions so that they can be used in sets for simultaneous hybridization; this highly increases the usability of these probes and results in a significant gain in time and labour. Evidently, when other hybridization conditions should be preferred,

all probes should be adapted accordingly by adding or deleting a number of nucleotides at their extremities. It should be understood that these concommitant adaptations should give rise to essentially the same result, namely that the respective probes still hybridize specifically with the defined target. Such adaptations might also be necessary if the amplified material should be RNA in nature and not DNA as in the case for the NASBA system.

The hybridization conditions can be monitored relying upon several parameters, such as the nature and concentration of the components of the media, and the temperatures under which the hybrids are formed and washed.

The hybridization and wash temperature is limited in upper value depending on the sequence of the probe (its nucleic acid composition, kind and length). The maximum hybridization or wash temperature of the probes described in the present invention ranges from 40°C to 60°C, more preferably from 45°C to 55°C, in the specific hybridization and wash media as described in the Examples section. At higher temperatures duplexing (= formation of the hybrids) competes with the dissociation (or denaturation) of the hybrid formed between the probe and the target.

In a preferred hybridization medium of the invention, containing 3 x SSC and 20% formamide, hybridization temperatures can range from 45°C to 55°C, with a preferred hybridization temperature of 50°C. A preferred wash medium contains 3 x SSC and 20% formamide, and preferred wash temperatures are the same as the preferred hybridization temperatures, i.e. preferably between 45°C and 55°C, and most preferably 50°C.

However, when modifications are introduced, be it either in the probes or in the media, the temperatures at which the probes can be used to obtain the required specificity should be changed according to known relationships, such as those described in the following reference: Hames B and Higgins S (eds.). Nucleic acid hybridization. A practical approach, IRL Press, Oxford, U.K., 1985.

The selected nucleic acid probes derived from the 16S-23S rRNA spacer region and described by the present invention are listed in Table 1a (SEQ ID NO 1 to 64, 175 to 191, 193 to 201, and 210 to 212). As described in the examples section, some of these probes show a better sensitivity and/or specificity than others, and the better probes are therefore preferentially used in methods to detect the organism of interest in a biological sample. However, it is possible that for certain applications (e.g. epidemiology, substrain typing, ...) a set of probes including the less specific and/or less sensitive probes may be very

informative (see e.g. example 7).

The following definitions serve to illustrate the terms and expressions used in the different embodiments of the present invention as set out below.

The term "spacer" is an abbreviated term referring to the 16S-23S rRNA internal transcribed spacer region.

The term "probe" refers to single stranded sequence-specific oligonucleotides which have a sequence which is sufficiently complementary to hybridize to the target sequence to be detected.

The more specific term "spacer probe" refers to a probe as defined above having a sequence which is sufficiently complementary to hybridize to a target sequence which is located in the spacer region(s) of the organism (or group of organisms) to be detected.

Preferably said probes are 70%, 80%, 90%, or more than 95% homologous to the exact complement of the target sequence to be detected. These target sequences are either genomic DNA or precursor RNA, or amplified versions thereof.

Preferably, these probes are about 5 to 50 nucleotides long, more preferably from about 10 to 25 nucleotides. The nucleotides as used in the present invention may be ribonucleotides, deoxyribonucleotides and modified nucleotides such as inosine or nucleotides containing modified groups which do not essentially alter their hybridization characteristics. Moreover, it is obvious to the man skilled in the art that any of the below-specified probes can be used as such, or in their complementary form, or in their RNA form (wherein T is replaced by U).

The probes according to the invention can be formed by cloning of recombinant plasmids containing inserts including the corresponding nucleotide sequences, if need be by cleaving the latter out from the cloned plasmids upon using the adequate nucleases and recovering them, e.g. by fractionation according to molecular weight. The probes according to the present invention can also be synthesized chemically, for instance by the conventional phospho-triester method.

The term "complementary" nucleic acids as used herein means that the nucleic acid sequences can form a perfect base-paired double helix with each other.

The term "homologous" as used in the current application is synonymous for identical: this means that polynucleic acids which are said to be e.g. 80% homologous show 80% identical base pairs in the same position upon alignment of the sequences.

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The term "polynucleic acid" corresponds to either double-stranded or single-stranded cDNA or genomic DNA or RNA, containing at least 10, 20, 30, 40 or 50 contiguous nucleotides. A polynucleic acid which is smaller than 100 nucleotides in length is often also referred to as an oligonucleotide. Single stranded polynucleic acid sequences are always represented in the current invention from the 5' end to the 3' end.

The term 'closest neighbour' means the taxon which is known or expected to be most closely related in terms of DNA homology and which has to be differentiated from the organism of interest.

The expression 'desired hybridization characteristics' means that the probe only hybridizes to the DNA or RNA from organisms for which it was designed, and not to DNA or RNA from other organisms (closest neighbours or organisms liable to be present in the same sample). In practice, this means that the intensity of the hybridization signal is at least two, three, four, five, ten or more times stronger with the target DNA or RNA from the organisms for which the probes were designed, as compared to non-target sequences.

These desired hybridization characteristics correspond to what is called later in the text "specific hybridization".

The expression "taxon-specific hybridization" or "taxon-specific probe" means that the probe only hybridizes to the DNA or RNA from the taxon for which it was designed and not to DNA or RNA from other taxa.

The term taxon can refer to a complete genus or a sub-group within a genus, a species or even subtype within a species (subspecies, serovars, sequevars, biovars...).

The term "specific amplification" or "specific primers" refers to the fact that said primers only amplify the spacer region from these organisms for which they were designed, and not from other organisms.

The term "sensitivity" refers to the number of false negatives: i.e. if 1 of the 100 strains to be detected is missed out, the test shows a sensitivity of (100-1/100)% = 99%.

The term "specificity" refers to the number of false positives: i.e. if on 100 strains detected, 2 seem to belong to organisms for which the test is not designed, the specificity of the test is (100-2/100)% = 98%.

The probes selected as being "preferential" show a sensitivity and specificity of more than 80%, preferably more than 90% and most preferably more than 95%.

The term "primer" refers to a single stranded DNA oligonucleotide sequence capable

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of acting as a point of initiation for synthesis of a primer extension product which is complementary to the nucleic acid strand to be copied. The length and the sequence of the primer must be such that they allow to prime the synthesis of the extension products. Preferably the primer is about 5-50 nucleotides long. Specific length and sequence will depend on the complexity of the required DNA or RNA targets, as well as on the conditions of primer use such as temperature and ionic strenght. The fact that amplification primers do not have to match exactly with the corresponding template sequence to warrant proper amplification is amply documented in the literature (Kwok et al., 1990).

The amplification method used can be either polymerase chain reaction (PCR; Saiki et al., 1988), ligase chain reaction (LCR; Landgren et al., 1988; Wu & Wallace, 1989; Barany, 1991), nucleic acid sequence-based amplification (NASBA; Guatelli et al., 1990; Compton. 1991), transcription-based amplification system (TAS; Kwoh et al., 1989), strand displacement amplification (SDA; Duck, 1990; Walker et al., 1992) or amplification by means of Qβ replicase (Lizardi et al., 1988; Lomeli et al., 1989) or any other suitable method to amplify nucleic acid molecules known in the art.

The oligonucleotides used as primers or probes may also comprise nucleotide analogues such as phosphorothioates (Matsukura et al., 1987), alkylphosphorothioates (Miller et al., 1979) or peptide nucleic acids (Nielsen et al., 1991; Nielsen et al., 1993) or may contain intercalating agents (Asseline et al., 1984).

As most other variations or modifications introduced into the original DNA sequences of the invention these variations will necessitate adaptions with respect to the conditions under which the oligonucleotide should be used to obtain the required specificity and sensitivity. However the eventual results of hybridisation will be essentially the same as those obtained with the unmodified oligonucleotides.

The introduction of these modifications may be advantageous in order to positively influence characteristics such as hybridization kinetics, reversibility of the hybrid-formation, biological stability of the oligonucleotide molecules, etc.

The term "solid support" can refer to any substrate to which an oligonucleotide probe can be coupled, provided that it retains its hybridization characteristics and provided that the background level of hybridization remains low. Usually the solid substrate will be a microtiter plate, a membrane (e.g. nylon or nitrocellulose) or a microsphere (bead). Prior to application to the membrane or fixation it may be convenient to modify the nucleic acid

probe in order to facilitate fixation or improve the hybridization efficiency. Such modifications may encompass homopolymer tailing, coupling with different reactive groups such as aliphatic groups, NH_2 groups, SH groups, carboxylic groups, or coupling with biotin, haptens or proteins.

The term "labelled" refers to the use of labelled nucleic acids. Labelling may be carried out by the use of labelled nucleotides incorporated during the polymerase step of the amplification such as illustrated by Saiki et al. (1988) or Bej et al. (1990) or by the use of labelled primers, or by any other method known to the person skilled in the art. The nature of the label may be isotopic (^{32}P, ^{35}S, etc.) or non-isotopic (biotin, digoxigenin, etc.).

The "sample" may be any biological material taken either directly from the infected human being (or animal), or after culturing (enrichment), or a sample taken from food or feed. Biological material may be e.g. expectorations of any kind, broncheolavages, blood, skin tissue, biopsies, lymphocyte blood culture material, colonies, etc. Said samples may be prepared or extracted according to any of the techniques known in the art.

The "target" material in these samples may be either genomic DNA or precursor RNA of the organism to be detected (= target organism), or amplified versions thereof as set out above. More specifically, the nucleic acid sequence of the target material is localized in the spacer region of the target organism(s).

Detection and identification of the target material can be performed by using one of the many electrophoresis methods, hybridization methods or sequencing methods described in literature and currently known by men skilled in the art. However, a very convenient and advantageous technique for the simultaneous detection of nucleic acids possibly present in biological samples is the Line Probe Assay technique. The Line Probe Assay (LiPA) is a reverse hybridization format (Saiki et al., 1989) using membrane strips onto which several oligonucleotide probes (including negative or positive control oligonucleotides) can be conveniently applied as parallel lines.

The LiPA technique, as described by Stuyver et al. (1993) and in international application WO 94/12670, provides a very rapid and user-friendly hybridization test. Results can be read within 4 h. after the start of the amplification. After amplification during which usually a non-isotopic label is incorporated in the amplified product, and alkaline denaturation, the amplified product is contacted with the probes on the membrane and the hybridization is carried out for about 1 to 1,5 h. Consequently, the hybrids formed are

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detected by an enzymatic procedure resulting in a visual purple-brown precipitate. The LiPA format is completely compatible with commercially availabe scanning devices, thus rendering automatic interpretation of the results possible. All those advantages make the LiPA format liable for use in a routine setting.

The LiPA format is not only an advantageous tool for identification and detection of pathogens at the species level but also at higher or lower taxonomical levels. For instance, probe-configurations on LiPA strips can be selected in such a manner that they can detect a complete genus (e.g. Neisseria, Listeria, etc.) or can identify subgroups within a genus (e.g. subgroups in the Mycobacterium avium-intracellulare-scrofulaceum complex) or can in some cases even detect subtypes (subspecies, serovars, sequevars, biovars, etc. whatever is clinically relevant) within a species.

It should be stressed that the ability to simultaneously generate hybridization results with a number of probes is an outstanding benefit of the LiPA technology. In many cases the amount of information which can be obtained by a particular combination of probes greatly outnumbers the data obtained by using single probe assays. Therefor the selection of probes on the membrane strip is of utmost importance since an optimized set of probes will generate the maximum of information possible. This is more particularly exemplified further herein.

The fact that different probes can be combined on one strip also offers the possibility to conveniently cope with a lack of sensitivity due to sequence heterogenity in the target region of the group of organisms to be detected. Due to this heterogenity, two or more probes may be required to positively identify all organisms of the particular group. These probes can be applied to membrane strips at different locations and the result is interpreted as positive if at least one of these probes is positive. Alternatively these probes can be applied as a mixture at the same location, hereby reducing the number of lines on a strip. This reduction may be convenient in order to make the strip more concise or to be able to extend the total number of probes on one strip. Another alternative approach, in view of its practical benefits, is the synthesis of oligonucleotides harbouring the sequences of two (or more) different probes (=degenerate probes) which then can be further processed and applied to the strip as one oligonucleotide molecule. This approach would considerably simplify the manufacturing procedures of the LiPA-strips. For example, probes with nucleotide sequences A and B are both required to detect all strains of taxon X. In the latter alternative a probe can be synthesized having the nucleotide sequence AB. This probe will have the combined

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characteristics of probes A and B.

By virtue of the above-mentioned properties the LiPA system can be considered as a preferential format for a hybridization method wherein several organisms need to be detected simultaneously in a sample. Moreover, as described in the examples section, the LiPA system is a preferred format for a selection method for the experimental evaluation and selection of theoretically designed probes.

However, it should be clear that any other hybridization assay, whereby different probes are used under the same hybridization and wash conditions can be used for the above-mentioned detection and/or selection methods. For example, it may be possible to immobilize the target nucleic acid to a solid support, and use mixtures of different probes, all differently labeled, resulting in a different detection signal for each of the probes hybridized to the target.

As an example, the procedure to be followed for the detection of one or more organisms in a sample using the LiPA format is outlined below :

- First, the nucleic acids of the organism(s) to be detected in the sample, is made available for amplification and/or hybridization.

- Secondly, the nucleic acids, if present, are amplified with one or another target amplification system, as specified below. Usually, amplification is needed to enhance the subsequent hybridization signal. However for some samples or some organisms amplification might not be necessary. This might also be the case if, for the detection of the hybrids formed, highly sensitive signal-amplification systems are used.

- Thirdly, eventually after a denaturation step, the nucleic acids present in the sample or the resulting amplified product are contacted with LiPA strips onto which one or more DNA-probes, allowing the detection of the organisms of interest, are immobilized, and hybridization is allowed to proceed.

- Finally, eventually after having performed a wash step, the hybrids are detected using a convenient and compatible detection system. From the hybridization signals or patterns observed the presence or absence of one or several organisms screened for in that particular biological sample can be deduced.

The amplification system used may be more or less universal, depending on the specific application needed.

By using universal primers located in the conserved flanking regions (16S and 23S

gene) of the rRNA spacer, the spacer region of most if not all organisms of eubacterial origin will be amplified. The same result may be obtained by using a combination of different sets of primers with reduced universality (multiplex amplification, i.e. an amplification procedure in which two or more primer sets are used simultaneously in one and the same reaction mixture).

For some applications it may be appropiate to amplify not all organisms present in the sample but more specifically, beforehand defined taxa. This may be achieved using specific primers located either in less conserved parts of the flanking genes of the spacers (e.g. MYCP1-5 for amplification of the spacer region of mycobacteria) or located in the spacers themselves (e.g. LIS-P1-P7 , BRU-P1-4, CHTR-P1-2 and YEC-P1-2 for specific amplification of the spacer region(s) of Listeria species, Brucella species, Chlamydia trachomatis, and Yersinia enterocolitica respectively).

The present invention thus provides a method for detection and identification of at least one micro-organism, or for the simultaneous detection of several micro-organisms in a sample, comprising the steps of:

(i)   if need be releasing, isolating and/or concentrating the polynucleic acids from the micro-organism(s) to be detected in the sample;

(ii)   if need be amplifying the 16S-23S rRNA spacer region, or a part of it, from the micro-organism(s) to be detected, with at least one suitable primer pair;

(iii)   hybridizing the polynucleic acids of step (i) or (ii) with a set of probes comprising at least two probes, under the same hybridization and wash conditions, with said probes being selected from the sequences of table 1a or equivalents thereof and/or from taxon-specific probes derived from any of the spacer sequences represented in figs. 1-103, with said taxon-specific probe being selected such that it is capable of hybridizing under the same hybridization and wash conditions as at least one of the probes of table 1a;

(iv)   detecting the hybrids formed in step (iii);

(v)   identification of the micro-organism(s) present in the sample from the differential hybridization signals obtained in step (iv).

The probes as mentioned in table 1a are all selected in such a way that they show the desired hybridization characteristics at a hybridization and wash temperature of 50°C in a preferred hybridization and wash medium of 3X SSC and 20% formamide.

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The term "equivalents" of a probe, also called "variants" or "homologues" or "obvious derivatives", refers to probes differing in sequence from any of the probes specified in table 1 either by addition to or removal from any of their respective extremities of one or several nucleotides, or by changing one or more nucleotides within said sequences, or a combination of both, provided that said equivalents still hybridize with the same RNA or DNA target as the corresponding unmodified probe sequence. Said equivalents share at least 75% homology, preferably more than 80%, most preferably more than 85% homology with the corresponding unmodified probe sequence. It should be noted that, when using an equivalent of a probe, it may be necessary to modify the hybridization conditions to obtain the same specificity as the corresponding unmodified probe. As a consequence, since it is the aim of this invention to use a set of probes which work under the same hybridization and wash conditions, it will also be necessary to modify accordingly the sequence of the other probes, belonging to the same set as the original unmodified probe. These modifications can be done according to principles known in the art, e.g. such as those described in Hames B and Higgins S (Eds): Nucleic acid hybridization. Practical approach. IRL Press, Oxford, UK, 1985.

The invention also provides for a method to select taxon-specific probes from the spacer region sequence(s) of said taxon, said probes being selected such that they show their desired hybridization characteristics under unified hybridization and wash conditions.

The term "unified" conditions means that these conditions are the same for the different probes enabling the detection of different taxa.

Preferentially, the present invention provides for a method as described above wherein at least 2 micro-organisms are detected simultaneously.

In a preferred embodiment, the set of probes as described in step (iii) is comprising at least two probes being selected from the sequences of table 1a, or equivalents thereof.

In another embodiment, the set of probes as described in step (iii) is comprising at least one probe being selected from the sequences of table 1a, or equivalents thereof, and at least one taxon-specific probe derived from any of the spacer sequences as represented in figs. 1-103.

In still another embodiment, the set of probes as described in step (iii) is comprising at least two taxon-specific probes derived from any of the spacer sequences as represented in figs. 1-103.

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The present invention also provides for a method as described above, wherein the probes as specified in step (iii) are combined with at least one other probe, preferentially also from the 16S-23S rRNA spacer region, enabling the simultaneous detection of different pathogenic bacteria liable to be present in the same sample.

The organisms of clinical relevance present in biological samples may vary considerably depending on the origin of the sample. The most common pathogenic bacteria which may be found in sputum samples, or in samples originating from the respiratory tract, are :

- Moraxella catarrhalis
- Streptococcus pneumomiae
- Haemophilus influenzae
- Pseudomonas aeruginosa
- Mycoplasma pneumomiae
- Acinetobacter species
- Mycobacterium species
- Staphylococcus aureus
- Legionella pneumophila

A LiPA-strip harbouring spacer-probes enabling the detection of most if not all of these organisms would be extremely benificial for reasons explained above.

Evidently, this also applies for other biological samples, as there are :
cerebrospinal fluid, urogenital samples, gastrointestinal samples, blood, urine, food products, soil, etc. For example, a preferred panel for cerebrospinal fluid would contain probe combinations enabling the detection and differentiation of the following organisms :

- Neisseria meningitidis
- Streptococcus pneumoniae
- Streptococcus agalactiae
- Listeria monocytogenes
- Mycobacterium tuberculosis

For some of the above mentioned organisms, spacer probes were already designed in a previous patent application (WO 91/16454). In order to be able to detect most pathogens possibly present in a sample in a single test, the probes of the present invention may be combined with at least one of the probes of WO 91/16454, or their obvious derivatives as

specified in WO 91/16454. For clarity, these probes are listed hereafter:

<u>Neisseria gonorrheoae</u>:   NGI1:   CGATGCGTCGTTATTCTACTTCGC

                                NGI2:   TTCGTTTACCTACCCGTTGACTAAGTAAGCAAAC

<u>Neisseria meningitidis</u>:   NMI1:   GGTCAAGTGTGACGTCGCCCTG

                                NMI2:   GTTCTTGGTCAAGTGTGACGTC

                                NMI3:   GCGTTCGTTATAGCTATCTACTGTGC

                                NMI4:   TGCGTTCGATATTGCTATCTACTGTGCA

                                NMI5:   TTTTGTTCTTGGTCAAGTGTGACGTCGCCCTGAA
                                        TGGATTCTGTTCCATT

                                NMI6:   TTTGCCTAACATTCCGTTGACTAGAACATCAGAC

<u>Haemophilus ducreyi</u>   HDI1:   TTATTATGCGCGAGGCATATTG

<u>Branhamella catharralis</u>   BCI1:   TTAAACATCTTACCAAAG

                                BCI2:   TTGATGTTTAAACTTGCTTGGTGGA

<u>Bordetella pertussis</u>   BPI1:   CCACACCCATCCTCTGGACAGGCTT

<u>Haemophilus influenzae</u>   HII1:   ACGCATCAAATTGACCGCACTT

                                HII2:   ACTTTGAAGTGAAAACTTAAAG

<u>Streptococcus agalactiae</u> SAI1:   AATCGAAAGGTTCAAATTGTT

                                SAI2:   GGAAACCTGCCATTTGCGTCTT

                                SAI3:   TCCACGATCTAGAAATAGATTGTAGAA

                                SAI4:   TCTAGTTTTAAAGAAACTAGGTT

<u>Streptococcus pneumoniae</u> SPI1:   GTGAGAGATCACCAAGTAATGCA

                                SPI2:   AGGAACTGCGCATTGGTCTT

                                SPI3:   GAGTTTATGACTGAAAGGTCAGAA

The invention thus provides for a method as described above, wherein said sample is originating from the respiratory tract, and wherein the set of probes as defined in step (iii) comprises at least one probe chosen from the following spacer probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)
MAI-ICG-1 :	CAACAGCAAATGATTGCCAGACACAC	(SEQ ID NO 6)

MIL-ICG-11 :   GAGGGGTTCCCGTCTGTAGTG   (SEQ ID NO 7)

MIL-ICG-22 :   TGAGGGGTTCTCGTCTGTAGTG   (SEQ ID NO 8)

MAC-ICG-1 :   CACTCGGTCGATCCGTGTGGA   (SEQ ID NO 9)

MAV-ICG-1 :   TCGGTCCGTCCGTGTGGAGTC   (SEQ ID NO 10)

MAV-ICG-22 :   GTGGCCGGCGTTCATCGAAA   (SEQ ID NO 11)

MIN-ICG-1 :   GCATAGTCCTTAGGGCTGATGCGTT   (SEQ ID NO 12)

MIN-ICG-2 :   GCTGATGCGTTCGTCGAAATGTGTA   (SEQ ID NO 13)

MIN-ICG-22 :   CTGATGCGTTCGTCGAAATGTGT   (SEQ ID NO 14)

MIN-ICG-222 : TGATGCGTTCGTCGAAATGTGT   (SEQ ID NO 15)

MIN-ICG-2222 :   GGCTGATGCGTTCGTCGAAATGTGTAA   (SEQ ID NO 16)

MAL-ICG-1 :   ACTAGATGAACGCGTAGTCCTTGT   (SEQ ID NO 17)

MHEF-ICG-1 :   TGGACGAAAACCGGGTGCACAA   (SEQ ID NO 18)

MAH-ICG-1 :   GTGTAATTTCTTTTTAACTCTTGTGTGTAAGTAAGTG

                                                (SEQ ID NO 19)

MCO-ICG-11 :   TGGCCGGCGTGTTCATCGAAA   (SEQ ID NO 20)

MTH-ICG-11 :   GCACTTCAATTGGTGAAGTGCGAGCC   (SEQ ID NO 21)

MTH-ICG-2 :   GCGTGGTCTTCATGGCCGG   (SEQ ID NO 22)

MEF-ICG-11 :   ACGCGTGGTCCTTCGTGG   (SEQ ID NO 23)

MSC-ICG-1 :   TCGGCTCGTTCTGAGTGGTGTC   (SEQ ID NO 24)

MKA-ICG-1 :   GATGCGTTTGCTACGGGTAGCGT   (SEQ ID NO 25)

MKA-ICG-2 :   GATGCGTTGCCTACGGGTAGCGT   (SEQ ID NO 26)

MKA-ICG-3 :   ATGCGTTGCCCTACGGGTAGCGT   (SEQ ID NO 27)

MKA-ICG-4 :   CGGGCTCTGTTCGAGAGTTGTC   (SEQ ID NO 28)

MKA-ICG-5 :   CCCTCAGGGATTTTCTGGGTGTTG   (SEQ ID NO 182)

MKA-ICG-6 :   GGACTCGTCCAAGAGTGTTGTCC   (SEQ ID NO 183)

MKA-ICG-7 :   TCGGGCTTGGCCAGAGCTGTT   (SEQ ID NO 184)

MKA-ICG-8 :   GGGTGCGCAACAGCAAGCGA   (SEQ ID NO 185)

MKA-ICG-9 :   GATGCGTTGCCCCTACGGG   (SEQ ID NO 186)

MKA-ICG-10 :   CCCTACGGGTAGCGTGTTCTTTTG   (SEQ ID NO 187)

MCH-ICG-1 :   GGTGTGGACTTTGACTTCTGAATAG   (SEQ ID NO 29)

MCH-ICG-2 :   CGGCAAAACGTCGGACTGTCA   (SEQ ID NO 30)

MCH-ICG-3 :   GGTGTGGTCCTTGACTTATGGATAG   (SEQ ID NO 210)

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MGO-ICG-1 :	AACACCCTCGGGTGCTGTCC	(SEQ ID NO 31)
MGO-ICG-2 :	GTATGCGTTGTCGTTCGCGGC	(SEQ ID NO 32)
MGO-ICG-5 :	CGTGAGGGGTCATCGTCTGTAG	(SEQ ID NO 33)
MUL-ICG-1 :	GGTTTCGGGATGTTGTCCCACC	(SEQ ID NO 175)
MGV-ICG-1 :	CGACTGAGGTCGACGTGGTGT	(SEQ ID NO 176)
MGV-ICG-2 :	GGTGTTTGAGCATTGAATAGTGGTTGC	(SEQ ID NO 177)
MGV-ICG-3 :	TCGGGCCGCGTGTTCGTCAAA	(SEQ ID NO 211)
MXE-ICG-1 :	GTTGGGCAGCAGGCAGTAACC	(SEQ ID NO 178)
MSI-ICG-1 :	CCGGCAACGGTTACGTGTTC	(SEQ ID NO 179)
MFO-ICG-1 :	TCGTTGGATGGCCTCGCACCT	(SEQ ID NO 180)
MFO-ICG-2 :	ACTTGGCGTGGGATGCGGGAA	(SEQ ID NO 181)
MML-ICG-1 :	CGGATCGATTGAGTGCTTGTCCC	(SEQ ID NO 188)
MML-ICG-2 :	TCTAAATGAACGCACTGCCGATGG	(SEQ ID NO 189)
MCE-ICG-1 :	TGAGGGAGCCCGTGCCTGTA	(SEQ ID NO 190)
MHP-ICG-1 :	CATGTTGGGCTTGATCGGGTGC	(SEQ ID NO 191)
PA-ICG 1 :	TGGTGTGCTGCGTGATCCGAT	(SEQ ID NO 34)
PA-ICG 2 :	TGAATGTTCGTGGATGAACATTGATT	(SEQ ID NO 35)
PA-ICG 3 :	CACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 36)
PA-ICG 4 :	TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	
		(SEQ ID NO 37)
PA-ICG 5 :	CTCTTTCACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 38)
MPN-ICG 1 :	ATCGGTGGTAAATTAAACCCAAATCCCTGT	(SEQ ID NO 49)
MPN-ICG 2 :	CAGTTCTGAAAGAACATTTCCGCTTCTTTC	(SEQ ID NO 50)
MGE-ICG 1 :	CACCCATTAATTTTTTCGGTGTTAAAACCC	(SEQ ID NO 51)
Mycoplasma-ICG :	CAAAACTGAAAACGACAATCTTTCTAGTTCC	(SEQ ID NO 52)
STAU-ICG 1 :	TACCAAGCAAAACCGAGTGAATAAAGAGTT	(SEQ ID NO 53)
STAU-ICG 2 :	CAGAAGATGCGGAATAACGTGAC	(SEQ ID NO 54)
STAU-ICG 3 :	AACGAAGCCGTATGTGAGCATTTGAC	(SEQ ID NO 55)
STAU-ICG 4 :	GAACGTAACTTCATGTTAACGTTTGACTTAT	(SEQ ID NO 56)
ACI-ICG 1 :	GCTTAAGTGCACAGTGCTCTAAACTGA	(SEQ ID NO 57)
ACI-ICG 2 :	CACGGTAATTAGTGTGATCTGACGAAG	(SEQ ID NO 58)

and more preferably from the following spacer probes:

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MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)
MAI-ICG-1 :	CAACAGCAAATGATTGCCAGACACAC	(SEQ ID NO 6)
MIL-ICG-11 :	GAGGGGTTCCCGTCTGTAGTG	(SEQ ID NO 7)
MIL-ICG-22 :	TGAGGGGTTCTCGTCTGTAGTG	(SEQ ID NO 8)
MAC-ICG-1 :	CACTCGGTCGATCCGTGTGGA	(SEQ ID NO 9)
MAV-ICG-1 :	TCGGTCCGTCCGTGTGGAGTC	(SEQ ID NO 10)
MAV-ICG-22 :	GTGGCCGGCGTTCATCGAAA	(SEQ ID NO 11)
MIN-ICG-1 :	GCATAGTCCTTAGGGCTGATGCGTT	(SEQ ID NO 12)
MAL-ICG-1 :	ACTAGATGAACGCGTAGTCCTTGT	(SEQ ID NO 17)
MCO-ICG-11 :	TGGCCGGCGTGTTCATCGAAA	(SEQ ID NO 20)
MTH-ICG-11 :	GCACTTCAATTGGTGAAGTGCGAGCC	(SEQ ID NO 21)
MTH-ICG-2 :	GCGTGGTCTTCATGGCCGG	(SEQ ID NO 22)
MEF-ICG-11 :	ACGCGTGGTCCTTCGTGG	(SEQ ID NO 23)
MSC-ICG-1 :	TCGGCTCGTTCTGAGTGGTGTC	(SEQ ID NO 24)
MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTCGAGAGTTGTC	(SEQ ID NO 28)
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)
MCH-ICG-3 :	GGTGTGGTCCTTGACTTATGGATAG	(SEQ ID NO 210)
MGO-ICG-5 :	CGTGAGGGGTCATCGTCTGTAG	(SEQ ID NO 33)
MUL-ICG-1 :	GGTTTCGGGATGTTGTCCCACC	(SEQ ID NO 175)
MGV-ICG-1 :	CGACTGAGGTCGACGTGGTGT	(SEQ ID NO 176)

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MGV-ICG-2 :	GGTGTTTGAGCATTGAATAGTGGTTGC	(SEQ ID NO 177)
MGV-ICG-3 :	TCGGGCCGCGTGTTCGTCAAA	(SEQ ID NO 211)
MXE-ICG-1 :	GTTGGGCAGCAGGCAGTAACC	(SEQ ID NO 178)
MSI-ICG-1 :	CCGGCAACGGTTACGTGTTC	(SEQ ID NO 179)
MFO-ICG-1 :	TCGTTGGATGGCCTCGCACCT	(SEQ ID NO 180)
MFO-ICG-2 :	ACTTGGCGTGGGATGCGGGAA	(SEQ ID NO 181)
MML-ICG-1 :	CGGATCGATTGAGTGCTTGTCCC	(SEQ ID NO 188)
MML-ICG-2 :	TCTAAATGAACGCACTGCCGATGG	(SEQ ID NO 189)
MCE-ICG-1 :	TGAGGGAGCCCGTGCCTGTA	(SEQ ID NO 190)
MHP-ICG-1 :	CATGTTGGGCTTGATCGGGTGC	(SEQ ID NO 191)

PA-ICG 1 :	TGGTGTGCTGCGTGATCCGAT	(SEQ ID NO 34)
PA-ICG 4 :	TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	
		(SEQ ID NO 37)
PA-ICG 5 :	CTCTTTCACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 38)
MPN-ICG 1 :	ATCGGTGGTAAATTAAACCCAAATCCCTGT	(SEQ ID NO 49)
MPN-ICG 2 :	CAGTTCTGAAAGAACATTTCCGCTTCTTTC	(SEQ ID NO 50)
MGE-ICG 1 :	CACCCATTAATTTTTTCGGTGTTAAAACCC	(SEQ ID NO 51)
Mycoplasma-ICG :	CAAAACTGAAAACGACAATCTTTCTAGTTCC	(SEQ ID NO 52)
STAU-ICG 1 :	TACCAAGCAAAACCGAGTGAATAAAGAGTT	(SEQ ID NO 53)
STAU-ICG 2 :	CAGAAGATGCGGAATAACGTGAC	(SEQ ID NO 54)
STAU-ICG 3 :	AACGAAGCCGTATGTGAGCATTTGAC	(SEQ ID NO 55)
STAU-ICG 4 :	GAACGTAACTTCATGTTAACGTTTGACTTAT	(SEQ ID NO 56)
ACI-ICG 1 :	GCTTAAGTGCACAGTGCTCTAAACTGA	(SEQ ID NO 57)
ACI-ICG 2 :	CACGGTAATTAGTGTGATCTGACGAAG	(SEQ ID NO 58)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 76 to 106, 157 to 174, 124, 125, 111 to 115, 139 to 144, or 126 to 130, and with said probes or equivalents being possibly used in combination with any probe detecting at least one of the following organisms: Haemophilus influenzae, Streptococcus

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pneumoniae, Moraxella catarrhalis or Bordetella pertussis.

The above mentioned probes of the invention are designed for the detection of
Mycobacterium species (SEQ ID NO 1 to 33 and 175 to 191), of Pseudomonas aeruginosa
(SEQ ID NO 34 to 38), of Mycoplasma species (SEQ ID NO 49 to 52), of Staphylococcus
aureus (SEQ ID NO 53 to 56) and of Acinetobacter baumanii (SEQ ID NO 57 and 58).

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes
are used simultaneously.

The invention also relates to a method as described above, wherein said sample is a
sample taken from the cerebrospinal fluid, and wherein the set of probes as described in step
(iii) comprises at least one probe chosen from the following spacer probes:

MYC-ICG-1 :   ACTGGATAGTGGTTGCGAGCATCTA   (SEQ ID NO 1)

MYC-ICG-22 :   CTTCTGAATAGTGGTTGCGAGCATCT   (SEQ ID NO 2)

MTB-ICG-1 :   GGGTGCATGACAACAAAGTTGGCCA   (SEQ ID NO 3)

MTB-ICG-2 :   GACTTGTTCCAGGTGTTGTCCCAC   (SEQ ID NO 4)

MTB-ICG-3 :   CGGCTAGCGGTGGCGTGTTCT   (SEQ ID NO 5)

LIS-ICG 1 :   CAAGTAACCGAGAATCATCTGAAAGTGAATC   (SEQ ID NO 39)

LMO-ICG 1 :   AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG

                                                       (SEQ ID NO 40)

LMO-ICG 2 :   TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC   (SEQ ID NO 41)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

LISP-ICG 1:   CGTTTTCATAAGCGATCGCACGTT   (SEQ ID NO 212)

and preferably from the following spacer probes:

MYC-ICG-1 :   ACTGGATAGTGGTTGCGAGCATCTA   (SEQ ID NO 1)

MYC-ICG-22 :   CTTCTGAATAGTGGTTGCGAGCATCT   (SEQ ID NO 2)

MTB-ICG-1 :   GGGTGCATGACAACAAAGTTGGCCA   (SEQ ID NO 3)

MTB-ICG-2 :   GACTTGTTCCAGGTGTTGTCCCAC   (SEQ ID NO 4)

MTB-ICG-3 :   CGGCTAGCGGTGGCGTGTTCT   (SEQ ID NO 5)

LIS-ICG 1 :   CAAGTAACCGAGAATCATCTGAAAGTGAATC   (SEQ ID NO 39)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

LISP-ICG 1:   CGTTTTCATAAGCGATCGCACGTT   (SEQ ID NO 212)

or equivalents of said probes,

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and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 116, 118-121, or 213-215,

and with said probes or equivalents being possibly used in combination with any probe detecting at least one of the following organisms: Neisseria meningitidis, Haemophilus influenzae or Streptococcus pneumoniae.

The above mentioned probes of the invention are designed for the detection of Mycobacterium species, and more particularly Mycobacterium tuberculosis (SEQ ID NO 1 to 5), and of Listeria species, more particularly Listeria monocytogenes (SEQ ID NO 39 to 42).

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

The invention also relates to a method as described above, wherein said sample is a sample taken from the urogenital tract, and wherein the set of probes as described in step (iii) comprises at least one probe chosen from the following spacer probes:

CHTR-ICG 1 :	GGAAGAAGCCTGAGAAGGTTTCTGAC	(SEQ ID NO 45)
CHTR-ICG 2 :	GCATTTATATGTAAGAGCAAGCATTCTATTTCA	(SEQ ID NO 46)
CHTR-ICG 3 :	GAGTAGCGTGGTGAGGACGAGA	(SEQ ID NO 47)
CHTR-ICG 4 :	GAGTAGCGCGGTGAGGACGAGA	(SEQ ID NO 201)
CHPS-ICG 1 :	GGATAACTGTCTTAGGACGGTTTGAC	(SEQ ID NO 48)
MGE-ICG 1 :	CACCCATTAATTTTTTCGGTGTTAAAACCC	(SEQ ID NO 51)
Mycoplasma-ICG :	CAAAACTGAAAACGACAATCTTTCTAGTTCC	(SEQ ID NO 52)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 122, 123, 197, 124 or 125,

with said probes or equivalents being possibly used in combination with any probe detecting at least one of the following organisms: Neisseria gonorrhoeae, Haemophilus ducreyi or Streptococcus agalactiae.

The above mentioned probes of the invention are designed for the detection of

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<u>Chlamydia</u> species (SEQ ID NO 45 to 48 and 201) and of <u>Mycoplasma</u> species (SEQ ID NO 51 and 52).

Preferentially, at least two, three, four, five, six or seven of said probes are used simultaneously.

The invention also relates to a method as described above, wherein said sample is a sample taken from food, and wherein the set of probes as defined in step (iii) comprises at least one probe chosen from the following spacer probes:

LIS-ICG 1 :   CAAGTAACCGAGAATCATCTGAAAGTGAATC   (SEQ ID NO 39)

LMO-ICG 1 :   AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG

(SEQ ID NO 40)

LMO-ICG 2 :   TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC   (SEQ ID NO 41)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

LIV-ICG 1 :   GTTAGCATAAATAGGTAACTATTTATGACACAAGTAAC

(SEQ ID NO 43)

LSE-ICG 1 :AGTTAGCATAAGTAGTGTAACTATTTATGACACAAG (SEQ ID NO 44)

LISP-ICG 1:   CGTTTTCATAAGCGATCGCACGTT   (SEQ ID NO 212)

STAU-ICG 1 :   TACCAAGCAAAACCGAGTGAATAAAGAGTT   (SEQ ID NO 53)

STAU-ICG 2 :   CAGAAGATGCGGAATAACGTGAC   (SEQ ID NO 54)

STAU-ICG 3 :   AACGAAGCCGTATGTGAGCATTTGAC   (SEQ ID NO 55)

STAU-ICG 4 :   GAACGTAACTTCATGTTAACGTTTGACTTAT   (SEQ ID NO 56)

BRU-ICG 1 :   CGTGCCGCCTTCGTTTCTCTTT   (SEQ ID NO 59)

BRU-ICG 2 :   TTCGCTTCGGGGTGGATCTGTG   (SEQ ID NO 60)

BRU-ICG 3 :   GCGTAGTAGCGTTTGCGTCGG   (SEQ ID NO 193)

BRU-ICG 4 :   CGCAAGAAGCTTGCTCAAGCC   (SEQ ID NO 194)

SALM-ICG 1 :   CAAAACTGACTTACGAGTCACGTTTGAG   (SEQ ID NO 61)

SALM-ICG 2 :   GATGTATGCTTCGTTATTCCACGCC   (SEQ ID NO 62)

STY-ICG 1 :   GGTCAAACCTCCAGGGACGCC   (SEQ ID NO 63)

SED-ICG 1 :   GCGGTAATGTGTGAAAGCGTTGCC   (SEQ ID NO 64)

YEC-ICG 1 :   GGAAAAGGTACTGCACGTGACTG   (SEQ ID NO 198)

YEC-ICG 2 :   GACAGCTGAAACTTATCCCTCCG   (SEQ ID NO 199)

YEC-ICG 3 :   GCTACCTGTTGATGTAATGAGTCAC   (SEQ ID NO 200)

and preferably from the following spacer probes:

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LIS-ICG 1 :	CAAGTAACCGAGAATCATCTGAAAGTGAATC	(SEQ ID NO 39)
LMO-ICG 3 :	AGGCACTATGCTTGAAGCATCGC	(SEQ ID NO 42)
LISP-ICG 1:	CGTTTTCATAAGCGATCGCACGTT	(SEQ ID NO 212)
STAU-ICG 1 :	TACCAAGCAAAACCGAGTGAATAAAGAGTT	(SEQ ID NO 53)
STAU-ICG 2 :	CAGAAGATGCGGAATAACGTGAC	(SEQ ID NO 54)
STAU-ICG 3 :	AACGAAGCCGTATGTGAGCATTTGAC	(SEQ ID NO 55)
STAU-ICG 4 :	GAACGTAACTTCATGTTAACGTTTGACTTAT	(SEQ ID NO 56)
BRU-ICG 2 :	TTCGCTTCGGGGTGGATCTGTG	(SEQ ID NO 60)
BRU-ICG 3 :	GCGTAGTAGCGTTTGCGTCGG	(SEQ ID NO 193)
BRU-ICG 4 :	CGCAAGAAGCTTGCTCAAGCC	(SEQ ID NO 194)
SALM-ICG 1 :	CAAAACTGACTTACGAGTCACGTTTGAG	(SEQ ID NO 61)
YEC-ICG 1 :	GGAAAAGGTACTGCACGTGACTG	(SEQ ID NO 198)
YEC-ICG 2 :	GACAGCTGAAACTTATCCCTCCG	(SEQ ID NO 199)
YEC-ICG 3 :	GCTACCTGTTGATGTAATGAGTCAC	(SEQ ID NO 200)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 116, 118-121, 213-215, 139-144, 131, 132, 154, 133-138, 195 or 196, with said probes or equivalents being possibly used in combination with any probe detecting strains of Campylobacter species.

The above mentioned probes of the invention are designed for the detection of Listeria species (SEQ ID NO 39 to 44), of Staphylococcus species (SEQ ID NO 53 to 56), of Brucella species (SEQ ID NO 59, 60, 193 and 194), of Salmonella species (SEQ ID NO 61 to 64) and of Yersinia enterocolitica (SEQ ID NO 198 to 200).

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

The invention also relates to a method as described above, wherein said sample is a sample taken from the gastrointestinal tract of a patient, and wherein the set of probes as defined in step (iii) comprises at least one probe chosen from the following spacer probes:

| SALM-ICG 1 : | CAAAACTGACTTACGAGTCACGTTTGAG | (SEQ ID NO 61) |
| SALM-ICG 2 : | GATGTATGCTTCGTTATTCCACGCC | (SEQ ID NO 62) |

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STY-ICG 1 :	GGTCAAACCTCCAGGGACGCC	(SEQ ID NO 63)
SED-ICG 1 :	GCGGTAATGTGTGAAAGCGTTGCC	(SEQ ID NO 64)
YEC-ICG 1 :	GGAAAAGGTACTGCACGTGACTG	(SEQ ID NO 198)
YEC-ICG 2 :	GACAGCTGAAACTTATCCCTCCG	(SEQ ID NO 199)
YEC-ICG 3 :	.GCTACCTGTTGATGTAATGAGTCAC	(SEQ ID NO 200)

and preferably from the following spacer probes:

SALM-ICG 1 :	CAAAACTGACTTACGAGTCACGTTTGAG	(SEQ ID NO 61)
YEC-ICG 1 :	GGAAAAGGTACTGCACGTGACTG	(SEQ ID NO 198)
YEC-ICG 2 :	GACAGCTGAAACTTATCCCTCCG	(SEQ ID NO 199)
YEC-ICG 3 :	GCTACCTGTTGATGTAATGAGTCAC	(SEQ ID NO 200)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 133-138 or 195-196,

with said probes or equivalents being possibly used in combination with any probe detecting Campylobacter species.

The above mentioned probes of the invention are designed to detect Salmonella species (SEQ ID NO 61 to 64) and Yersinia enterocolitica (SEQ ID NO 198 to 200).

Preferentially, at least two, three, four, five, six or seven of said probes are used simultaneously.

The invention also relates to the use of the selected probes or their equivalents for the detection of specific bacterial taxa, said taxa being either a complete genus, or a subgroup within a genus, a species, or even a subtype within a species.

The invention thus provides for a method as described above to detect and identify one or more strains of Mycobacterium species and subspecies in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)

MAI-ICG-1 :	CAACAGCAAATGATTGCCAGACACAC	(SEQ ID NO 6)
MIL-ICG-11 :	GAGGGGTTCCCGTCTGTAGTG	(SEQ ID NO 7)
MIL-ICG-22 :	TGAGGGGTTCTCGTCTGTAGTG	(SEQ ID NO 8)
MAC-ICG-1 :	CACTCGGTCGATCCGTGTGGA	(SEQ ID NO 9)
MAV-ICG-1 :	TCGGTCCGTCCGTGTGGAGTC	(SEQ ID NO 10)
MAV-ICG-22 :	GTGGCCGGCGTTCATCGAAA	(SEQ ID NO 11)
MIN-ICG-1 :	GCATAGTCCTTAGGGCTGATGCGTT	(SEQ ID NO 12)
MIN-ICG-2 :	GCTGATGCGTTCGTCGAAATGTGTA	(SEQ ID NO 13)
MIN-ICG-22 :	CTGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 14)
MIN-ICG-222 :	TGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 15)
MIN-ICG-2222 :	GGCTGATGCGTTCGTCGAAATGTGTAA	(SEQ ID NO 16)
MAL-ICG-1 :	ACTAGATGAACGCGTAGTCCTTGT	(SEQ ID NO 17)
MHEF-ICG-1 :	TGGACGAAAACCGGGTGCACAA	(SEQ ID NO 18)
MAH-ICG-1 :	GTGTAATTTCTTTTTTAACTCTTGTGTGTAAGTAAGTG	
		(SEQ ID NO 19)
MCO-ICG-11 :	TGGCCGGCGTGTTCATCGAAA	(SEQ ID NO 20)
MTH-ICG-11 :	GCACTTCAATTGGTGAAGTGCGAGCC	(SEQ ID NO 21)
MTH-ICG-2 :	GCGTGGTCTTCATGGCCGG	(SEQ ID NO 22)
MEF-ICG-11 :	ACGCGTGGTCCTTCGTGG	(SEQ ID NO 23)
MSC-ICG-1 :	TCGGCTCGTTCTGAGTGGTGTC	(SEQ ID NO 24)
MKA-ICG-1 :	GATGCGTTTGCTACGGGTAGCGT	(SEQ ID NO 25)
MKA-ICG-2 :	GATGCGTTGCCTACGGGTAGCGT	(SEQ ID NO 26)
MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTCGAGAGTTGTC	(SEQ ID NO 28)
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)

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MGO-ICG-1 :   AACACCCTCGGGTGCTGTCC   (SEQ ID NO 31)

MGO-ICG-2 :   GTATGCGTTGTCGTTCGCGGC   (SEQ ID NO 32)

MGO-ICG-5 :   CGTGAGGGGTCATCGTCTGTAG   (SEQ ID NO 33)

MUL-ICG-1 :   GGTTTCGGGATGTTGTCCCACC   (SEQ ID NO 175)

MGV-ICG-1 :   CGACTGAGGTCGACGTGGTGT   (SEQ ID NO 176)

MGV-ICG-2 :   GGTGTTTGAGCATTGAATAGTGGTTGC   (SEQ ID NO 177)

MXE-ICG-1 :   GTTGGGCAGCAGGCAGTAACC   (SEQ ID NO 178)

MSI-ICG-1 : CCGGCAACGGTTACGTGTTC   (SEQ ID NO 179)

MFO-ICG-1 :   TCGTTGGATGGCCTCGCACCT   (SEQ ID NO 180)

MFO-ICG-2 :   ACTTGGCGTGGGATGCGGGAA   (SEQ ID NO 181)

MML-ICG-1 :   CGGATCGATTGAGTGCTTGTCCC   (SEQ ID NO 188)

MML-ICG-2 :   TCTAAATGAACGCACTGCCGATGG   (SEQ ID NO 189)

MCE-ICG-1 :   TGAGGGAGCCCGTGCCTGTA   (SEQ ID NO 190)

MHP-ICG-1 :   CATGTTGGGCTTGATCGGGTGC   (SEQ ID NO 191)

and more preferably to at least one probe of the following restricted group of spacer probes:

MYC-ICG-1 :   ACTGGATAGTGGTTGCGAGCATCTA   (SEQ ID NO 1)

MYC-ICG-22 :   CTTCTGAATAGTGGTTGCGAGCATCT   (SEQ ID NO 2)

MTB-ICG-1 :   GGGTGCATGACAACAAAGTTGGCCA   (SEQ ID NO 3)

MTB-ICG-2 :   GACTTGTTCCAGGTGTTGTCCCAC   (SEQ ID NO 4)

MTB-ICG-3 :   CGGCTAGCGGTGGCGTGTTCT   (SEQ ID NO 5)

MAI-ICG-1 :   CAACAGCAAATGATTGCCAGACACAC   (SEQ ID NO 6)

MIL-ICG-11 :   GAGGGGTTCCCGTCTGTAGTG   (SEQ ID NO 7)

MIL-ICG-22 :   TGAGGGGTTCTCGTCTGTAGTG   (SEQ ID NO 8)

MAC-ICG-1 :   CACTCGGTCGATCCGTGTGGA   (SEQ ID NO 9)

MAV-ICG-1 :   TCGGTCCGTCCGTGTGGAGTC   (SEQ ID NO 10)

MAV-ICG-22 :   GTGGCCGGCGTTCATCGAAA   (SEQ ID NO 11)

MIN-ICG-1 :   GCATAGTCCTTAGGGCTGATGCGTT   (SEQ ID NO 12)

MAL-ICG-1 :   ACTAGATGAACGCGTAGTCCTTGT   (SEQ ID NO 17)

MCO-ICG-11 :   TGGCCGGCGTGTTCATCGAAA   (SEQ ID NO 20)

MTH-ICG-11 :   GCACTTCAATTGGTGAAGTGCGAGCC   (SEQ ID NO 21)

MTH-ICG-2 :   GCGTGGTCTTCATGGCCGG   (SEQ ID NO 22)

MEF-ICG-11 :   ACGCGTGGTCCTTCGTGG   (SEQ ID NO 23)

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MSC-ICG-1 :	TCGGCTCGTTCTGAGTGGTGTC	(SEQ ID NO 24)
MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTCGAGAGTTGTC	(SEQ ID NO 28)
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)
MCH-ICG-3 :	GGTGTGGTCCTTGACTTATGGATAG	(SEQ ID NO 210)
MGO-ICG-5 :	CGTGAGGGGTCATCGTCTGTAG	(SEQ ID NO 33)
MUL-ICG-1 :	GGTTTCGGGATGTTGTCCCACC	(SEQ ID NO 175)
MGV-ICG-1 :	CGACTGAGGTCGACGTGGTGT	(SEQ ID NO 176)
MGV-ICG-2 :	GGTGTTTGAGCATTGAATAGTGGTTGC	(SEQ ID NO 177)
MGV-ICG-3 :	TCGGGCCGCGTGTTCGTCAAA	(SEQ ID NO 211)
MXE-ICG-1 :	GTTGGGCAGCAGGCAGTAACC	(SEQ ID NO 178)
MSI-ICG-1 :	CCGGCAACGGTTACGTGTTC	(SEQ ID NO 179)
MFO-ICG-1 :	TCGTTGGATGGCCTCGCACCT	(SEQ ID NO 180)
MFO-ICG-2 :	ACTTGGCGTGGGATGCGGGAA	(SEQ ID NO 181)
MML-ICG-1 :	CGGATCGATTGAGTGCTTGTCCC	(SEQ ID NO 188)
MML-ICG-2 :	TCTAAATGAACGCACTGCCGATGG	(SEQ ID NO 189)
MCE-ICG-1 :	TGAGGGAGCCCGTGCCTGTA	(SEQ ID NO 190)
MHP-ICG-1 :	CATGTTGGGCTTGATCGGGTGC	(SEQ ID NO 191)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 76-110, or 157-174 provided said probe hybridizes specifically to a <u>Mycobacterium</u> species.

The sequences represented by SEQ ID NO 76-110 and 157-174 are new.

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

As described above, the preferred restricted set of probes are those probes which

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showed a sensitivity and specificity of more than 80%, preferably more than 90%, most preferably more than 95%, under the specific hybridization conditions as described in the examples section.

In one specific embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycobacterium</u> <u>tuberculosis</u> complex strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MTB-ICG-1 :   GGGTGCATGACAACAAAGTTGGCCA   (SEQ ID NO 3)

MTB-ICG-2 :   GACTTGTTCCAGGTGTTGTCCCAC   (SEQ ID NO 4)

MTB-ICG-3 :   CGGCTAGCGGTGGCGTGTTCT   (SEQ ID NO 5)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 76 provided said probe hybridizes specifically to the <u>M</u>. <u>tuberculosis</u> complex. The <u>M</u>. <u>tuberculosis</u> complex comprises <u>M</u>. <u>tuberculosis</u>, <u>M</u>. <u>bovis</u>, <u>M</u>. <u>bovis</u> BCG, <u>M</u>. <u>africanum</u> and <u>M</u>. <u>microti</u> strains.

The sequence represented in SEQ ID NO 76 is new.

Preferentially, at least two, or three of said probes are used simultaneously.

In another specific embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycobacterium</u> strains from the MAIS-complex, wherein step (iii) comprises hybridizing to at least one of the following probes:

MAI-ICG-1 :   CAACAGCAAATGATTGCCAGACACAC   (SEQ ID NO 6)

MIL-ICG-11 :   GAGGGGTTCCCGTCTGTAGTG   (SEQ ID NO 7)

MIL-ICG-22 :   TGAGGGGTTCTCGTCTGTAGTG   (SEQ ID NO 8)

MAC-ICG-1 :   CACTCGGTCGATCCGTGTGGA   (SEQ ID NO 9)

MAV-ICG-1 :   TCGGTCCGTCCGTGTGGAGTC   (SEQ ID NO 10)

MAV-ICG-22 :   GTGGCCGGCGTTCATCGAAA   (SEQ ID NO 11)

MIN-ICG-1 :   GCATAGTCCTTAGGGCTGATGCGTT   (SEQ ID NO 12)

MIN-ICG-2 :   GCTGATGCGTTCGTCGAAATGTGTA   (SEQ ID NO 13)

MIN-ICG-22 :   CTGATGCGTTCGTCGAAATGTGT   (SEQ ID NO 14)

MIN-ICG-222 : TGATGCGTTCGTCGAAATGTGT   (SEQ ID NO 15)

MIN-ICG-2222 : GGCTGATGCGTTCGTCGAAATGTGTAA   (SEQ ID NO 16)

MAL-ICG-1 :   ACTAGATGAACGCGTAGTCCTTGT   (SEQ ID NO 17)

MHEF-ICG-1 :   TGGACGAAAACCGGGTGCACAA   (SEQ ID NO 18)

MAH-ICG-1 :   GTGTAATTTCTTTTTTAACTCTTGTGTGTAAGTAAGTG

                                                                        (SEQ ID NO 19)
MCO-ICG-11 : TGGCCGGCGTGTTCATCGAAA   (SEQ ID NO 20)
MTH-ICG-11 : GCACTTCAATTGGTGAAGTGCGAGCC   (SEQ ID NO 21)
MTH-ICG-2 :   GCGTGGTCTTCATGGCCGG   (SEQ ID NO 22)
MEF-ICG-11 : ACGCGTGGTCCTTCGTGG   (SEQ ID NO 23)
MSC-ICG-1 :   TCGGCTCGTTCTGAGTGGTGTC   (SEQ ID NO 24)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 77-100 or 108-110, provided said probe hybridizes specifically to strains from the MAIS complex. The MAIS complex as defined in this invention comprises all strains of M. avium, M. intracellulare and M. scrofulaceum and all strains closely related to the above mentioned species and not clearly belonging to another defined Mycobacterium species. The latter group of strains are defined in this invention as "MIC strains" (M. intracellulare complex).

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

In still another specific embodiment, the invention provides for a method as described above, to detect and identify one or more M. avium and M. paratuberculosis strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MAV-ICG-1 :   TCGGTCCGTCCGTGTGGAGTC   (SEQ ID NO 10)
MAV-ICG-22 : GTGGCCGGCGTTCATCGAAA   (SEQ ID NO 11)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 77 and 78 provided said probe hybridizes specifically to M. avium or M. paratuberculosis.

The sequences as represented in SEQ ID NO 77 and 78 are new.

Preferentially, this embodiment uses both probes in combination.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium intracellulare strains and MIC-strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MAI-ICG-1 :   CAACAGCAAATGATTGCCAGACACAC   (SEQ ID NO 6)
MIL-ICG-11 : GAGGGGTTCCCGTCTGTAGTG   (SEQ ID NO 7)
MIL-ICG-22 : TGAGGGGTTCTCGTCTGTAGTG   (SEQ ID NO 8)

MAC-ICG-1 :   CACTCGGTCGATCCGTGTGGA   (SEQ ID NO 9)

MIN-ICG-1 :   GCATAGTCCTTAGGGCTGATGCGTT   (SEQ ID NO 12)

MIN-ICG-2 :   GCTGATGCGTTCGTCGAAATGTGTA   (SEQ ID NO 13)

MIN-ICG-22 :   CTGATGCGTTCGTCGAAATGTGT   (SEQ ID NO 14)

MIN-ICG-222 :   TGATGCGTTCGTCGAAATGTGT   (SEQ ID NO 15)

MIN-ICG-2222 : GGCTGATGCGTTCGTCGAAATGTGTAA   (SEQ ID NO 16)

MAL-ICG-1 :   ACTAGATGAACGCGTAGTCCTTGT   (SEQ ID NO 17)

MHEF-ICG-1 :   TGGACGAAAACCGGGTGCACAA   (SEQ ID NO 18)

MAH-ICG-1 :   GTGTAATTTCTTTTTTAACTCTTGTGTGTAAGTAAGTG

                                                           (SEQ ID NO 19)

MCO-ICG-11 :   TGGCCGGCGTGTTCATCGAAA   (SEQ ID NO 20)

MTH-ICG-11 :   GCACTTCAATTGGTGAAGTGCGAGCC   (SEQ ID NO 21)

MTH-ICG-2 :   GCGTGGTCTTCATGGCCGG   (SEQ ID NO 22)

MEF-ICG-11 :   ACGCGTGGTCCTTCGTGG   (SEQ ID NO 23)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 provided said probe hybridizes specifically to <u>M. intracellulare</u> strains and MIC-strains.

The sequences as represented in SEQ ID NO 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 are new.

Preferentially, at least two, three, four, five, six, seven, eight or more of said probes are used simultaneously.

In still another specific embodiment, the invention provides for a method as described above, to detect and identify one or more <u>Mycobacterium intracellulare</u> strains in a sample, wherein step (iii) comprises hybridizing to at least the following probes :

MIN-ICG-1 : GCATAGTCCTTAGGGCTGATGCGTT   (SEQ ID NO 12)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 89 provided said probe hybridizes specifically to <u>M. intracellulare</u> strains.

In still another specific embodiment, the invention provides for a method as described above, to detect and identify one or more <u>Mycobacterium scrofulaceum</u> strains in a sample, wherein step (iii) comprises hybrdizing to the following probe:

MSC-ICG-1 :   TCGGCTCGTTCTGAGTGGTGTC   (SEQ ID NO 24)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 100 provided said probe hybridizes specifically to M. scrofulaceum.

The sequence as represented in SEQ ID NO 100 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium kansasii strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MKA-ICG-1 :   GATGCGTTTGCTACGGGTAGCGT   (SEQ ID NO 25)

MKA-ICG-2 :   GATGCGTTGCCTACGGGTAGCGT   (SEQ ID NO 26)

MKA-ICG-3 :   ATGCGTTGCCCTACGGGTAGCGT   (SEQ ID NO 27)

MKA-ICG-4 :   CGGGCTCTGTTCGAGAGTTGTC   (SEQ ID NO 28)

MKA-ICG-5 :   CCCTCAGGGATTTTCTGGGTGTTG   (SEQ ID NO 182)

MKA-ICG-6 :   GGACTCGTCCAAGAGTGTTGTCC   (SEQ ID NO 183)

MKA-ICG-7 :   TCGGGCTTGGCCAGAGCTGTT   (SEQ ID NO 184)

MKA-ICG-8 :   GGGTGCGCAACAGCAAGCGA   (SEQ ID NO 185)

MKA-ICG-9 :   GATGCGTTGCCCCTACGGG   (SEQ ID NO 186)

MKA-ICG-10 :   CCCTACGGGTAGCGTGTTCTTTTG   (SEQ ID NO 187)

and more preferably to:

MKA-ICG-3 :   ATGCGTTGCCCTACGGGTAGCGT   (SEQ ID NO 27)

MKA-ICG-4 :   CGGGCTCTGTTCGAGAGTTGTC   (SEQ ID NO 28)

MKA-ICG-5 :   CCCTCAGGGATTTTCTGGGTGTTG   (SEQ ID NO 182)

MKA-ICG-6 :   GGACTCGTCCAAGAGTGTTGTCC   (SEQ ID NO 183)

MKA-ICG-7 :   TCGGGCTTGGCCAGAGCTGTT   (SEQ ID NO 184)

MKA-ICG-8 :   GGGTGCGCAACAGCAAGCGA   (SEQ ID NO 185)

MKA-ICG-9 :   GATGCGTTGCCCCTACGGG   (SEQ ID NO 186)

MKA-ICG-10 :   CCCTACGGGTAGCGTGTTCTTTTG   (SEQ ID NO 187)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 101, 167, 168 or 169 provided said probe hybridizes specifically to M. kansasii.

The sequences as represented in SEQ ID NO 101, 167, 168 and 169 are new.

Preferentially, at least two, three or four of said probes are used simultaneously.

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In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycobacterium</u> <u>chelonae</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MCH-ICG-1 :   GGTGTGGACTTTGACTTCTGAATAG   (SEQ ID NO 29)

MCH-ICG-2 :   CGGCAAAACGTCGGACTGTCA   (SEQ ID NO 30)

MCH-ICG-3 :   GGTGTGGTCCTTGACTTATGGATAG   (SEQ ID NO 210)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 102, 103 or 174 provided said probe hybridizes specifically to <u>M</u>. <u>chelonae</u>. According to another preferential embodiment, these three probes are used in combination.

The sequences as represented in SEQ ID NO 102, 103 and 174 are new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycobacterium</u> <u>gordonae</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MGO-ICG-1 :   AACACCCTCGGGTGCTGTCC   (SEQ ID NO 31)

MGO-ICG-2 :   GTATGCGTTGTCGTTCGCGGC   (SEQ ID NO 32)

MGO-ICG-5 :   CGTGAGGGGTCATCGTCTGTAG   (SEQ ID NO 33)

and more preferably to:

MGO-ICG-5 :   CGTGAGGGGTCATCGTCTGTAG   (SEQ ID NO 33)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 104, 105 or 106 provided said probe hybridizes specifically to <u>M</u>. <u>gordonae</u>.

The sequences as represented in SEQ ID NO 104 to 106 are new.

Preferentially, at least two or three of said probes are used simultaneously.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycobacterium</u> <u>ulcerans</u> strains or <u>Mycobacterium</u> <u>marinum</u> strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MUL-ICG-1 :   GGTTTCGGGATGTTGTCCCACC   (SEQ ID NO 175)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 157 provided said probe hybridizes specifically to <u>M</u>. <u>ulcerans</u> and <u>M</u>. <u>marinum</u>.

The sequence as represented in SEQ ID NO 157 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycobacterium genavense</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MGV-ICG-1 : CGACTGAGGTCGACGTGGTGT (SEQ ID NO 176)

MGV-ICG-2 : GGTGTTTGAGCATTGAATAGTGGTTGC (SEQ ID NO 177)

MGV-ICG-3 : TCGGGCCGCGTGTTCGTCAAA (SEQ ID NO 211)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 158, 159, 160, 161 or 162 provided said probe hybridizes specifically to <u>M. genavense</u>.

The sequences as represented in SEQ ID NO 158 to 162 are new.

As described in the examples, <u>M. genavense</u> includes <u>M. genavense</u> strains sensu strictu and a group of closely related strains called <u>M. simiae</u>-like. The former group of strains can be detected specifically with probe MGV-ICG-1 while the latter group hybridizes specifically with probe MGV-ICG-3. Probe MGV-ICG-2 detects both groups.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycobacterium xenopi</u> strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MXE-ICG-1 : GTTGGGCAGCAGGCAGTAACC (SEQ ID NO 178)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 163 provided said probe hybridizes specifically to <u>M. xenopi</u>.

The sequence as represented in SEQ ID NO 163 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycobacterium simiae</u> strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MSI-ICG-1 : CCGGCAACGGTTACGTGTTC (SEQ ID NO 179)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 164 or 165 provided said probe hybridizes specifically to <u>M. simiae</u>.

The sequence as represented in SEQ ID NO 164 or 165 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycobacterium fortuitum</u> strains in a sample,

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wherein step (iii) comprises hybridizing to at least one of the the following probes:

MFO-ICG-1 :   TCGTTGGATGGCCTCGCACCT   (SEQ ID NO 180)

MFO-ICG-2 :   ACTTGGCGTGGGATGCGGGAA   (SEQ ID NO 181)

or to equivalents of said probes or to any probe derived from SEQ ID NO 166 provided said probe hybridizes specifically to M. fortuitum.

The sequence as represented in SEQ ID NO 166 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium celatum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MCE-ICG-1 :   TGAGGGAGCCCGTGCCTGTA   (SEQ ID NO 190)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 170 provided said probe hybridizes specifically to M. celatum.

The sequence as represented in SEQ ID NO 170 is new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium haemophilum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MHP-ICG-1 :   CATGTTGGGCTTGATCGGGTGC   (SEQ ID NO 191)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 171, 172 or 173 provided said probe hybridizes specifically to M. haemophilum.

The sequences as represented in SEQ ID NO 171 to 173 are new.

In still another specific embodiment, the invention provides for a method as described above to detect and identify one or more Mycobacterium malmoense strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MML-ICG-1 :   CGGATCGATTGAGTGCTTGTCCC   (SEQ ID NO 188)

MML-ICG-2 :   TCTAAATGAACGCACTGCCGATGG   (SEQ ID NO 189)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 107 provided said probe hybridizes specifically to M. malmoense.

The sequence as represented in SEQ ID NO 107 is new.

In still another specific embodiment, the invention provides for a method as described

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above to detect and identify one or more <u>Mycobacterium</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MYC-ICG-1 :   ACTGGATAGTGGTTGCGAGCATCTA   . (SEQ ID NO 1)

MYC-ICG-22 :   CTTCTGAATAGTGGTTGCGAGCATCT   (SEQ ID NO 2)

or to equivalents of said probes.

According to a preferred embodiment, both probes are used in combination.

The invention also provides for a method as described above to detect and identify one or more <u>Mycoplasma</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MPN-ICG 1 :   ATCGGTGGTAAATTAAACCCAAATCCCTGT   (SEQ ID NO 49)

MPN-ICG 2 :   CAGTTCTGAAAGAACATTTCCGCTTCTTTC   (SEQ ID NO 50)

MGE-ICG 1 :   CACCCATTAATTTTTTCGGTGTTAAAACCC   (SEQ ID NO 51)

Mycoplasma-ICG :   CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID N0 124 or 125 provided said probe hybridizes specifically with <u>Mycoplasma</u> species.

Preferentially, at least two, three or four of said probes are used simultaneously.

More particularly, the invention provides for a method as described above to detect and identify one or more <u>Mycoplasma pneumoniae</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MPN-ICG 1 :   ATCGGTGGTAAATTAAACCCAAATCCCTGT   (SEQ ID NO 49)

MPN-ICG 2 :   CAGTTCTGAAAGAACATTTCCGCTTCTTTC   (SEQ ID NO 50)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 125 provided said probe hybridizes specifically to <u>Mycoplasma pneumoniae</u>. According to a preferred embodiment, both these probes are used in combination.

The sequence as represented in SEQ ID NO 125 is new.

In another particular embodiment, the invention provides for a method as described above to detect and identify one or more <u>Mycoplasma genitalium</u> strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MGE-ICG 1 :   CACCCATTAATTTTTTCGGTGTTAAAACCC   (SEQ ID NO 51)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 124 provided said probe hybridizes specifically to Mycoplasma genitalium.

The sequence as represented in SEQ ID NO 124 is new.

The invention also provides for a method as described above to detect and identify one or more Pseudomonas strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

PA-ICG 1 :   TGGTGTGCTGCGTGATCCGAT   (SEQ ID NO 34)

PA-ICG 2 :   TGAATGTTCGTGGATGAACATTGATT   (SEQ ID NO 35)

PA-ICG 3 :   CACTGGTGATCATTCAAGTCAAG   (SEQ ID NO 36)

PA-ICG 4 :   TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC

                                                          (SEQ ID NO 37)

PA-ICG 5 :   CTCTTTCACTGGTGATCATTCAAGTCAAG .   (SEQ ID NO 38)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 111, 112, 113, 114 or 115 provided said probe hybridizes specifically to Pseudomonas strains.

The sequneces as represented in SEQ ID NO 111 to 115 are new.

Preferentially, at least two, three or four of said probes are used simultaneously.

More particularly, the invention provides for a method as described above to detect and identify one or more Pseudomonas aeruginosa strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

PA-ICG 1 :   TGGTGTGCTGCGTGATCCGAT   (SEQ ID NO 34)

PA-ICG 2 :   TGAATGTTCGTGGATGAACATTGATT   (SEQ ID NO 35)

PA-ICG 3 :   CACTGGTGATCATTCAAGTCAAG   (SEQ ID NO 36)

PA-ICG 4 :   TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC

                                                          (SEQ ID NO 37)

PA-ICG 5 :   CTCTTTCACTGGTGATCATTCAAGTCAAG   (SEQ ID NO 38)

and most preferably to at least one of the following probes:

PA-ICG 1 :   TGGTGTGCTGCGTGATCCGAT   (SEQ ID NO 34)

PA-ICG 4 :   TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC

                                                          (SEQ ID NO 37)

PA-ICG 5 :   CTCTTTCACTGGTGATCATTCAAGTCAAG   (SEQ ID NO 38)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 111 provided said probe hybridizes specifically to Pseudomonas aeruginosa.

The sequence as represented in SEQ ID NO 111 is new.

Preferentially, at least two, three, four or five of said probes are used simultaneously.

The invention also provides for a method as described above to detect and identify one or more Staphylococcus species in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

STAU-ICG 1 :   TACCAAGCAAAACCGAGTGAATAAAGAGTT   (SEQ ID NO 53)

STAU-ICG 2 :   CAGAAGATGCGGAATAACGTGAC   (SEQ ID NO 54)

STAU-ICG 3 :   AACGAAGCCGTATGTGAGCATTTGAC   (SEQ ID NO 55)

STAU-ICG 4 :   GAACGTAACTTCATGTTAACGTTTGACTTAT   (SEQ ID NO 56)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 139, 140, 141, 142 ,143 or 144 provided said probe hybridizes specifically to Staphylococcus species.

The sequences as represented in SEQ ID NO 139 to 144 are new.

Preferentially, at least two, three or four of said probes are used simultaneously.

More particularly, the invention provides for a method as described above to detect and identify one or more Staphylococcus aureus strains in a sample, wherein step (iii) comprises hybridizing to at least one, and preferably both of the following probes:

STAU-ICG 3 :   AACGAAGCCGTATGTGAGCATTTGAC   (SEQ ID NO 55)

STAU-ICG 4 :   GAACGTAACTTCATGTTAACGTTTGACTTAT   (SEQ ID NO 56)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 139, 140, 141, 142 or 143 provided said probe hybridizes specifically to Staphylococcus aureus. According to a preferred embodiment, both these probes are used in combination.

In another specific embodiment the invention provides for a method as described above to detect and identify one or more Staphylococcus epidermidis strains in a sample, wherein step (iii) comprises hybrdizing to any probe derived from SEQ ID NO 144 as long as this probe can be caused to hybridize specifically to Staphylococcus epidermidis.

The invention also provides for a method as described above to detect and identify one or more Acinetobacter strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

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ACI-ICG 1 :   GCTTAAGTGCACAGTGCTCTAAACTGA   (SEQ ID NO 57)

ACI-ICG 2 :   CACGGTAATTAGTGTGATCTGACGAAG   (SEQ ID NO 58)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 126, 127, 128, 129 or 130 provided said probe hybridizes specifically to Acinetobacter sp.. According to a preferred embodiment, both these probes are used in combination.

The sequences as represented in SEQ ID NO 126 to 130 are new.

More particularly, the invention provides for a method as described above to detect and identify one or more Acinetobacter baumanii strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

ACI-ICG 1 :   GCTTAAGTGCACAGTGCTCTAAACTGA   (SEQ ID NO 57)

ACI-ICG 2 :   CACGGTAATTAGTGTGATCTGACGAAG   (SEQ ID NO 58)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 126 provided said probe hybridizes specifically to Acinetobacter baumanii. According to a preferred embodiment, both these probes are used in combination.

The invention also provides for a method as described above, to detect and identify one or more Listeria strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

LIS-ICG 1 :   CAAGTAACCGAGAATCATCTGAAAGTGAATC   (SEQ ID NO 39)

LMO-ICG 1 :   AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG

                                                    (SEQ ID NO 40)

LMO-ICG 2 :   TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC   (SEQ ID NO 41)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

LIV-ICG 1 :   GTTAGCATAAATAGGTAACTATTTATGACACAAGTAAC

                                                    (SEQ ID NO 43)

LSE-ICG 1 :   AGTTAGCATAAGTAGTGTAACTATTTATGACACAAG

LISP-ICG 1:   CGTTTTCATAAGCGATCGCACGTT   (SEQ ID NO 212)

and most preferably to at least one of the following probes:

LIS-ICG 1 :   CAAGTAACCGAGAATCATCTGAAAGTGAATC   (SEQ ID NO 39)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

LISP-ICG 1:   CGTTTTCATAAGCGATCGCACGTT   (SEQ ID NO 212)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 116, 118, 119, 120, 121, 213, 214 or 215 provided said probe hybridizes specifically to Listeria species.

As described in the examples section, Listeria species encompass Listeria species sensu strictu, and a group of closely related organisms referred to as "Listeria-like organisms". The latter group can be specifically recognized by probe LISP-ICG 1.

The sequences as represented in SEQ ID NO 116, 118 to 121 and 213 to 215 are new.

Preferentially, at least two, three, four, five or six of said probes are used simultaneously.

More particularly, the invention provides for a method as described above, to detect and identify one or more Listeria monocytogenes strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

LMO-ICG 1 :AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG(SEQ ID NO 40)

LMO-ICG 2 :   TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC   (SEQ ID NO 41)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

and most preferably to the following probe:

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 120 provided said probe hybridizes specifically to Listeria monocytogenes.

Preferentially, at least two, or three of said probes are used simultaneously.

The invention also provides for a method as described above to detect and identify one or more Brucella strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

BRU-ICG 1 :   CGTGCCGCCTTCGTTTCTCTTT   (SEQ ID NO 59)

BRU-ICG 2 :   TTCGCTTCGGGGTGGATCTGTG   (SEQ ID NO 60)

BRU-ICG 3 :   GCGTAGTAGCGTTTGCGTCGG   (SEQ ID NO 193)

BRU-ICG 4 :   CGCAAGAAGCTTGCTCAAGCC   (SEQ ID NO 194)

and most preferably to at least one of the following probes:

BRU-ICG 2 :   TTCGCTTCGGGGTGGATCTGTG   (SEQ ID NO 60)

BRU-ICG 3 :   GCGTAGTAGCGTTTGCGTCGG   (SEQ ID NO 193)

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BRU-ICG 4 :   CGCAAGAAGCTTGCTCAAGCC   (SEQ ID NO 194)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 131, 132 or 154 provided said probe hybridizes specifically to Brucella strains.

The sequences as represented in SEQ ID NO 131, 132 and 154 are new.

The invention also provides for a method as described above to detect and identify one or more Salmonella strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

SALM-ICG 1 :   CAAAACTGACTTACGAGTCACGTTTGAG   (SEQ ID NO 61)

SALM-ICG 2 :   GATGTATGCTTCGTTATTCCACGCC   (SEQ ID NO 62)

STY-ICG 1 :   GGTCAAACCTCCAGGGACGCC   (SEQ ID NO 63)

SED-ICG 1 :   GCGGTAATGTGTGAAAGCGTTGCC   (SEQ ID NO 64)

and most preferably to the following probe:

SALM-ICG 1 :   CAAAACTGACTTACGAGTCACGTTTGAG   (SEQ ID NO 61)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 133, 134, 135, 136, 137 or 138 provided said probe hybridizes specifically to Salmonella strains.

The sequences as represented in SEQ ID NO 133 to 138 are new.

Preferentially, at least two, three, or four of said probes are used simultaneously.

The invention also relates to a method as described above to detect and identify one or more Chlamydia strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

CHTR-ICG 1 :   GGAAGAAGCCTGAGAAGGTTTCTGAC   (SEQ ID NO 45)

CHTR-ICG 2 :   GCATTTATATGTAAGAGCAAGCATTCTATTTCA   (SEQ ID NO 46)

CHTR-ICG 3 :   GAGTAGCGTGGTGAGGACGAGA   (SEQ ID NO 47)

CHTR-ICG 4 :   GAGTAGCGCGGTGAGGACGAGA   (SEQ ID NO 201)

CHPS-ICG 1 :   GGATAACTGTCTTAGGACGGTTTGAC   (SEQ ID NO 48)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 122, 123 or 197 provided that said probe hybridizes specifically to Chlamydia strains.

Preferentially, at least two, three, four or five of said probes are used simultaneously.

More particularly, the invention relates to a method as described above to detect and

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identify one or more <u>Chlamydia</u> <u>trachomatis</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

CHTR-ICG 1 :   GGAAGAAGCCTGAGAAGGTTTCTGAC   (SEQ ID NO 45)

CHTR-ICG 2 :   GCATTTATATGTAAGAGCAAGCATTCTATTTCA   (SEQ ID NO 46)

CHTR-ICG 3 :   GAGTAGCGTGGTGAGGACGAGA   (SEQ ID NO 47)

CHTR-ICG 4 :   GAGTAGCGCGGTGAGGACGAGA   (SEQ ID NO 201)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 123 or 197 provided said probe hybridizes specifically to <u>Chlamydia</u> <u>trachomatis</u>.

The sequences as represented in SEQ ID NO 123 and 197 are new.

Preferentially, at least two, three or four of said probes are used simultaneously.

In another particular embodiment, the invention provides for a method as described above to detect and identify one or more <u>Chlamydia</u> <u>psittaci</u> strains in a sample, wherein step (iii) comprises hybridizing to at least the following probe:

CHPS-ICG 1 :   GGATAACTGTCTTAGGACGGTTTGAC   (SEQ ID NO 48)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 122 provided said probe hybridizes specifically to <u>Chlamydia</u> <u>psittaci</u>.

The sequence of SEQ ID NO 122 is new.

The invention also provides for a method as described above, to detect one or more <u>Streptococcus</u> strains in a sample, wherein step (iii) comprises hybridizing to any probe derived from SEQ ID NO 145, 146, 147, 148, 149, 150, 151, 152 or 153 provided said probe hybridizes specifically to <u>Streptococcus</u> strains, or equivalents of these probes.

The sequences as represented in SEQ ID NO 145, 146, 147, 148, 149, 150, 151, 152 or 153 are new.

The invention also provides for a method as described above, to detect one or more <u>Yersinia</u> <u>enterocolitica</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes :

YEC-ICG 1 : GGAAAAGGTACTGCACGTGACTG   (SEQ ID NO 198)

YEC-ICG 2 : GACAGCTGAAACTTATCCCTCCG   (SEQ ID NO 199)

YEC-ICG 3 : GCTACCTGTTGATGTAATGAGTCAC   (SEQ ID NO 200)

or to equivalents of said probes,

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and/or to any probe derived from SEQ ID NO 195 or 196, provided said probe hybridizes specifically to Yersinia enterocolitica.

The sequences as represented in SEQ ID NO 195 and 196 are new.

In some cases it may be advantageous to amplify not all organisms present in a sample, but only more specific taxa, which are considered to be relevant. In these cases the invention provides for primers allowing the specific amplification of the spacer region for only those beforehand defined taxa.

The invention thus provides for a method as described above to detect and identify specifically Chlamydia trachomatis in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers:

CHTR-P1 : AAGGTTTCTGACTAGGTTGGGC   (SEQ ID NO 69)

CHTR-P2 : GGTGAAGTGCTTGCATGGATCT   (SEQ ID NO 70)

or equivalents of these primers, said equivalents differing in sequence from the above mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region or part of it from Chlamydia trachomatis.

Preferably both primers are used.

The invention also provides for a method as described above to detect and identify specifically Listeria species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers:

LIS-P1 : ACCTGTGAGTTTTCGTTCTTCTC   (SEQ ID NO 71)

LIS-P2 : CTATTTGTTCAGTTTTGAGAGGTT   (SEQ ID NO 72)

LIS-P3 : ATTTTCCGTATCAGCGATGATAC   (SEQ ID NO 73)

LIS-P4 : ACGAAGTAAAGGTTGTTTTTCT   (SEQ ID NO 74)

LIS-P5 : GAGAGGTTACTCTCTTTTATGTCAG   (SEQ ID NO 75)

LIS-P6 : CTTTTATGTCAGATAAAGTATGCAA   (SEQ ID NO 202)

LIS-P7 : CGTAAAAGGGTATGATTATTTG   (SEQ ID NO 203)

or equivalents of these primers, said equivalents differing in sequence from the above mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region or part of it from Listeria species.

The invention also relates to a method as described above to detect and identify specifically Mycobacterium species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers:

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MYC-P1:   TCCCTTGTGGCCTGTGTG   (SEQ ID NO 65)

MYC-P2:   TCCTTCATCGGCTCTCGA   (SEQ ID NO 66)

MYC-P3:   GATGCCAAGGCATCCACC   (SEQ ID NO 67)

MYC-P4:   CCTCCCACGTCCTTCATCG   (SEQ ID NO 68)

MYC-P5:   CCTGGGTTTGACATGCACAG   (SEQ ID NO 192)

or equivalents of these primers, said equivalents differing in sequence from the above mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region or part of it from <u>Mycobacterium</u> species.

The invention also provides for a method as described above to detect and identify specifically <u>Brucella</u> species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or part of it, using at least one of the following primers :

BRU-P1 :   TCGAGAATTGGAAAGAGGTC   (SEQ ID NO 204)

BRU-P2 :   AAGAGGTCGGATTTATCCG   (SEQ ID NO 205)

BRU-P3 :   TTCGACTGCAAATGCTCG   (SEQ ID NO 206)

BRU-P4 :   TCTTAAAGCCGCATTATGC   (SEQ ID NO 207)

or equivalents of these primers, said equivalents differing in sequence from the above-mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region of part of it from <u>Brucella</u> species.

The invention also provides for a method as described above to detect and identify specifically <u>Yersinia enterocolitica</u> species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or part of it, using at least one of the following primers :

YEC-P1 :   CCTAATGATATTGATTCGCG   (SEQ ID NO 208)

YEC-P2 :   ATGACAGGTTAATCCTTACCCC   (SEQ ID NO 209)

or equivalents of these primers, said equivalents differing in sequence from the above-mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region of part of it from <u>Yersinia enterocolitica</u> species.

The invention also provides for a composition comprising at least one of the probes and/or primers as defined above.

Said composition may comprise any carrier, support, label or diluent known in the art for probes or primers, more particularly any of the labels or supports detailed in the definitions section.

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The invention relates more partcularly to isolated probes and primers as defined above, more particularly any of the probes as specified in Table 1a or any of the primers as specified in Table 1b.

According to another embodiment, the present invention relates also to new spacer region sequences as defined above and as set out in figures 1-103 (SEQ ID NO 76 to 154, SEQ ID NO 157 to 174, SEQ ID NO 195 to 197 and SEQ ID NO 213 to 215).

In another embodiment the invention provides for a reverse hybridization method comprising any of the probes as defined above, wherein said probes are immobilized on a known location on a solid support, more preferably on a membrane strip.

In yet another embodiment the invention provides for a kit for the detection and identification of at least one micro-organism, or the simultaneous detection and identification of several micro-organisms in a sample, comprising the following components:

(i)   when appropiate, at least one suitable primer pair to allow amplification of the intercistronic 16S-23S rRNA spacer region, or a part of it;

(ii)   at least one of the probes as defined above;

(iii)   a buffer, or components necessary to produce the buffer, enabling a hybridization reaction between said probes and the polynucleic acids present in the sample, or the amplified products thereof;

(iv)   a solution, or components necessary to produce the solution, enabling washing of the hybrids formed under the appropiate wash conditions;

(v)   when appropiate, a means for detecting the hybrids resulting from the preceding hybridization.

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FIGURE LEGENDS

Fig 1 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
               <u>Mycobacterium</u> <u>tuberculosis</u> strain H37RV ATCC 27294 (SEQ ID NO 76)

Fig 2 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
5   <u>Mycobacterium</u> <u>avium</u> ATCC 151.769 (ITG 4991) (SEQ ID NO 77)

Fig 3 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
               <u>Mycobacterium</u> <u>paratuberculosis</u> strains 316F and 2E (SEQ ID NO 78)

Fig 4 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
               <u>Mycobacterium</u> strain ITG 5513 (SEQ ID NO 79)

10   Fig 5 : represents the DNA sequence of the 16S-23S rRNA spacer region from
               <u>Mycobacterium</u> strain ITG 8695 (SEQ ID NO 80)

Fig 6 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
               <u>Mycobacterium</u> strain ITG 8708 (SEQ ID NO 81)

Fig 7 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
15   <u>Mycobacterium</u> strain ITG 8715 (SEQ ID NO 82)

Fig 8 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
               <u>Mycobacterium</u> strain ITG 8054 (SEQ ID NO 83)

Fig 9 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
               <u>Mycobacterium</u> strain ITG 8737 (SEQ ID NO 84)

20   Fig 10 : represents the DNA sequence of the 16S-23S rRNA spacer region from
               <u>Mycobacterium</u> strain ITG 8743 (SEQ ID NO 85)

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Fig 11 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
              Mycobacterium strain ITG 8745 (SEQ ID NO 86)

Fig 12 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
              Mycobacterium strain ITG 8748 (SEQ ID NO 87)

5   Fig 13 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
              Mycobacterium strain ITG 8752 (SEQ ID NO 88)

Fig 14 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
              Mycobacterium intracellulare serovar 12 ITG 5915 (SEQ ID NO 89)

Fig 15 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
10   Mycobacterium lufu ITG 4755 (SEQ ID NO 90)

Fig 16 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
              Mycobacterium strain ITG 5922 (SEQ ID NO 91)

Fig 17 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
              Mycobacterium strain ITG 1329 (SEQ ID NO 92)

15   Fig 18 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
              Mycobacterium strain ITG 1812 (SEQ ID NO 93)

Fig 19 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
              Mycobacterium strain ITG 5280 (SEQ ID NO 94)

Fig 20 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
20   Mycobacterium strain ITG 5620 (SEQ ID NO 95)

Fig 21 :   represents the DNA sequence of the 16S-23S rRNA spacer region from
              Mycobacterium strain ITG 5765 (SEQ ID NO 96)

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Fig 22 :　　　represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium ITG 7395 (SEQ ID NO 97)

Fig 23 :　　　represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium ITG 8738 (SEQ ID NO 98)

Fig 24 :　　　represents the DNA sequence of the 16S-23S rRNA spacer region from Mycobacterium ITG 926 (SEQ ID NO 99)

Fig 25 :　　　represents the DNA sequence of the 16S-23S spacer region from Mycobacterium scrofulaceum ITG 4988 (SEQ ID NO 100)

Fig 26 :　　　represents the DNA sequence of the 16S-23S spacer region from Mycobacterium kansasii ATCC 22478 (=ITG 4987) (SEQ ID NO 101)

Fig 27 :　　　represents the DNA sequence of the 16S-23S spacer region from Mycobacterium chelonae abcessus ITG 4975 (SEQ ID NO 102)

Fig 28 :　　　represents the DNA sequence of the 16S-23S spacer region from Mycobacterium chelonae chelonae ITG 9855 (SEQ ID NO 103)

Fig 29 :　　　represents the DNA sequence of the 16S-23S spacer region from Mycobacterium gordonae ITG 7703 (SEQ ID NO 104)

Fig 30 :　　　represents the DNA sequence of the 16S-23S spacer region from Mycobacterium gordonae ITG 7836 (SEQ ID NO 105)

Fig 31 :　　　represents the DNA sequence of the 16S-23S spacer region from Mycobacterium gordonae ITG 8059 (SEQ ID NO 106)

Fig 32 :　　　represents the DNA sequence of the 16S-23S spacer region from Mycobacterium malmoense ITG 4842 and ITG 4832 (SEQ ID NO 107)

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Fig 33 :   represents the DNA sequence of the 16S-23S spacer region from
            Mycobacterium strain 8757 (SEQ ID NO 108)

Fig 34 :   represents the DNA sequence of the 16S-23S spacer region from
            Mycobacterium ITG 8723 (SEQ ID NO 109)

5   Fig 35 :   represents the DNA sequence of the 16S-23S spacer region from
            Mycobacterium ITG 8724 (SEQ ID NO 110)

Fig 36 :   represents the DNA sequence of the 16S-23S spacer region from Pseudomonas
            aeruginosa UZG 5669 (SEQ ID NO 111)

Fig 37 :   represents the DNA sequence of the 16S-23S spacer region from Pseudomonas
10   pseudoalcaligenes LMG 1225 (SEQ ID NO 112)

Fig 38 :   represents the DNA sequence of the 16S-23S spacer region from Pseudomonas
            stutzeri LMG 2333 (SEQ ID NO 113)

Fig 39 :   represents the DNA sequence of the 16S-23S spacer region from Pseudomonas
            alcaligenes LMG 1224 (SEQ ID NO 114)

15 Fig 40 :   represents the DNA sequence of the 16S-23S spacer region from Pseudomonas
            putida LMG 2232 (SEQ ID NO 115)

Fig 41 :   represents the DNA sequence of the small 16S-23S spacer region from
            Listeria ivanovii CIP 7842 (SEQ ID NO 116)

Fig 42 :   represents the DNA sequence of the small 16S-23S spacer region from
20   Listeria monocytogenes (SEQ ID NO 117)

Fig 43 :   represents the DNA sequence of the small 16S-23S spacer region from
            Listeria seeligeri serovar 4A nr. 4268 (SEQ ID NO 118)

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Acinetobacter johnsonii LMG 999 (SEQ ID NO 129)

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Mycobacterium xenopi ITG 4986 (SEQ ID NO 163)

Fig 87 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium simiae A ITG 4485 (SEQ ID NO 164)

Fig 88 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium simiae B ITG 4484 (SEQ ID NO 165)

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Fig 89 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium fortuitum ITG 4304 (SEQ ID NO 166)

Fig 90 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium kansasii ITG 6328 (SEQ ID NO 167)

10 Fig 91 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium kansasii ITG 8698 (SEQ ID NO 168)

Fig 92 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium kansasii ITG 8973 (SEQ ID NO 169)

Fig 93 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium celatum ITG 94-123 (SEQ ID NO 170)

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Fig 94 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium haemophilum ITG 776 (SEQ ID NO 171)

Fig 95 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium haemophilum ITG 778 (SEQ ID NO 172)

20 Fig 96 : represents the DNA sequence of the 16S-23S spacer region from Mycobacterium haemophilum ITG 3071 (SEQ ID NO 173)

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TABLE LEGENDS

Table 1a: List of all new probes originating from the 16S-23S rRNA spacer region

Table 1b: List of possible primers to be used for taxon-specific amplification of the spacer region or part of it.

5   Table 2:   Hybridization results for Pseudomonas

Table 3:   Different probe patterns obtained for mycobacterial strain-types

Table 4:   Mycobacteria strains tested in LiPA

Table 5:   Hybridization results for Listeria (Probes LMO1, 2, LSE1, LIV1, LIS1)

Table 6:   Hybridization results for Listeria (Probes LMO3, LIS1)

10   Table 7:   Hybridization results for Chlamydia

Table 8:   New mycobacterial probes and hybridization results

Table 9:   Hybridization results for Brucella

Table 10:   Hybridization results for Staphylococcus

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<u>Table 1a</u>

PROBE	SEQUENCE	SEQ ID NO
MYC-ICG-1	: ACTGGATAGTGGTTGCGAGCATCTA	1
MYC-ICG-22	: CTTCTGAATAGTGGTTGCGAGCATCT	2
MTB-ICG-1	: GGGTGCATGACAACAAAGTTGGCCA	3
MTB-ICG-2	: GACTTGTTCCAGGTGTTGTCCCAC	4
MTB-ICG-3	: CGGCTAGCGGTGGCGTGTTCT	5
MAI-ICG-1	: CAACAGCAAATGATTGCCAGACACAC	6
MIL-ICG-11	: GAGGGGTTCCCGTCTGTAGTG	7
MIL-ICG-22	: TGAGGGGTTCTCGTCTGTAGTG	8
MAC-ICG-1	: CACTCGGTCGATCCGTGTGGA	9
MAV-ICG-1	: TCGGTCCGTCCGTGTGGAGTC	10
MAV-ICG-22	: GTGGCCGGCGTTCATCGAAA	11
MIN-ICG-1	: GCATAGTCCTTAGGGCTGATGCGTT	12
MIN-ICG-2	: GCTGATGCGTTCGTCGAAATGTGTA	13
MIN-ICG-22	: CTGATGCGTTCGTCGAAATGTGT	14
MIN-ICG-222	: TGATGCGTTCGTCGAAATGTGT	15
MIN-ICG-2222	: GGCTGATGCGTTCGTCGAAATGTGTAA	16
MAL-ICG-1	: ACTAGATGAACGCGTAGTCCTTGT	17
MHEF-ICG-1	: TGGACGAAAACCGGGTGCACAA	18
MAH-ICG-1	: GTGTAATTTCTTTTTTAACTCTTGTGTGTAAGTAAGTG	19
MCO-ICG-11	: TGGCCGGCGTGTTCATCGAAA	20
MTH-ICG-11	: GCACTTCAATTGGTGAAGTGCGAGCC	21
MTH-ICG-2	: GCGTGGTCTTCATGGCCGG	22
MEF-ICG-11	: ACGCGTGGTCCTTCGTGG	23
MSC-ICG-1	: TCGGCTCGTTCTGAGTGGTGTC	24
MKA-ICG-1	: GATGCGTTTGCTACGGGTAGCGT	25
MKA-ICG-2	: GATGCGTTGCCTACGGGTAGCGT	26
MKA-ICG-3	: ATGCGTTGCCCTACGGGTAGCGT	27
MKA-ICG-4	: CGGGCTCTGTTCGAGAGTTGTC	28
MCH-ICG-1	: GGTGTGGACTTTGACTTCTGAATAG	29
MCH-ICG-2	: CGGCAAAACGTCGGACTGTCA	30

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	MCH-ICG-3	: GGTGTGGTCCTTGACTTATGGATAG	210
	MGO-ICG-1	: AACACCCTCGGGTGCTGTCC	31
	MGO-ICG-2	: GTATGCGTTGTCGTTCGCGGC	32
	MGO-ICG-5	: CGTGAGGGGTCATCGTCTGTAG	33
5	MUL-ICG-1	: GGTTTCGGGATGTTGTCCCACC	175
	MGV-ICG-1	: CGACTGAGGTCGACGTGGTGT	176
	MGV-ICG-2	: GGTGTTTGAGCATTGAATAGTGGTTGC	177
	MGV-ICG-3	: TCGGGCCGCGTGTTCGTCAAA	211
	MXE-ICG-1	: GTTGGGCAGCAGGCAGTAACC	178
10	MSI-ICG-1	: CCGGCAACGGTTACGTGTTC	179
	MFO-ICG-1	: TCGTTGGATGGCCTCGCACCT	180
	MFO-ICG-2	: ACTTGGCGTGGGATGCGGGAA	181
	MKA-ICG-5	: CCCTCAGGGATTTTCTGGGTGTTG	182
	MKA-ICG-6	: GGACTCGTCCAAGAGTGTTGTCC	183
15	MKA-ICG-7	: TCGGGCTTGGCCAGAGCTGTT	184
	MKA-ICG-8	: GGGTGCGCAACAGCAAGCGA	185
	MKA-ICG-9	: GATGCGTTGCCCCTACGGG	186
	MKA-ICG-10	: CCCTACGGGTAGCGTGTTCTTTTG	187
	MML-ICG-1	: CGGATCGATTGAGTGCTTGTCCC	188
20	MML-ICG-2	: TCTAAATGAACGCACTGCCGATGG	189
	MCE-ICG-1	: TGAGGGAGCCCGTGCCTGTA	190
	MHP-ICG-1	: CATGTTGGGCTTGATCGGGTGC	191
	PA-ICG 1	: TGGTGTGCTGCGTGATCCGAT	34
	PA-ICG 2	: TGAATGTTCGTGGATGAACATTGATT	35
25	PA-ICG 3	: CACTGGTGATCATTCAAGTCAAG	36
	PA-ICG 4	: TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	37
	PA-ICG 5	: CTCTTTCACTGGTGATCATTCAAGTCAAG	38
	LIS-ICG 1	: CAAGTAACCGAGAATCATCTGAAAGTGAATC	39
	LMO-ICG 1	: AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG	40
30	LMO-ICG 2	: TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC	41
	LMO-ICG 3	: AGGCACTATGCTTGAAGCATCGC	42
	LIV-ICG 1	: GTTAGCATAAATAGGTAACTATTTATGACACAAGTAAC	43

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	LSE-ICG 1	: AGTTAGCATAAGTAGTGTAACTATTTATGACACAAG	44
	LISP-ICG 1	: CGTTTTCATAAGCGATCGCACGTT	212
	CHTR-ICG 1	: GGAAGAAGCCTGAGAAGGTTTCTGAC	45
	CHTR-ICG 2	: GCATTTATATGTAAGAGCAAGCATTCTATTTCA	46
5	CHTR-ICG 3	: GAGTAGCGTGGTGAGGACGAGA	47
	CHPS-ICG 1	: GGATAACTGTCTTAGGACGGTTTGAC	48
	MPN-ICG 1	: ATCGGTGGTAAATTAAACCCAAATCCCTGT	49
	MPN-ICG 2	: CAGTTCTGAAAGAACATTTCCGCTTCTTTC	50
	MGE-ICG 1	: CACCCATTAATTTTTTCGGTGTTAAAACCC	51
10	Mycoplasma-ICG	: CAAAACTGAAAACGACAATCTTTCTAGTTCC	52
	STAU-ICG 1	: TACCAAGCAAAACCGAGTGAATAAAGAGTT	53
	STAU-ICG 2	: CAGAAGATGCGGAATAACGTGAC	54
	STAU-ICG 3	: AACGAAGCCGTATGTGAGCATTTGAC	55
	STAU-ICG 4	: GAACGTAACTTCATGTTAACGTTTGACTTAT	56
15	ACI-ICG 1	: GCTTAAGTGCACAGTGCTCTAAACTGA	57
	ACI-ICG 2	: CACGGTAATTAGTGTGATCTGACGAAG	58
	BRU-ICG 1	: CGTGCCGCCTTCGTTTCTCTTT	59
	BRU-ICG 2	: TTCGCTTCGGGGTGGATCTGTG	60
	BRU-ICG 3	: GCGTAGTAGCGTTTGCGTCGG	193
20	BRU-ICG 4	: CGCAAGAAGCTTGCTCAAGCC	194
	SALM-ICG 1	: CAAAACTGACTTACGAGTCACGTTTGAG	61
	SALM-ICG 2	: GATGTATGCTTCGTTATTCCACGCC	62
	STY-ICG 1	: GGTCAAACCTCCAGGGACGCC	63
	SED-ICG 1	: GCGGTAATGTGTGAAAGCGTTGCC	64
25	YEC-ICG 1	: GGAAAAGGTACTGCACGTGACTG	198
	YEC-ICG 2	: GACAGCTGAAACTTATCCCTCCG	199
	YEC-ICG 3	: GCTACCTGTTGATGTAATGAGTCAC	200
	CHTR-ICG 4	: GAGTAGCGCGGTGAGGACGAGA	201

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Table 1b

	PRIMERS		SEQUENCE	SEQ ID NO
	MYC-P1	:	TCCCTTGTGGCCTGTGTG	65
	MYC-P2	:	TCCTTCATCGGCTCTCGA	66
5	MYC-P3	:	GATGCCAAGGCATCCACC	67
	MYC-P4	:	CCTCCCACGTCCTTCATCG	68
	MYC-P5	:	CCTGGGTTTGACATGCACAG	192
	CHTR-P1	:	AAGGTTTCTGACTAGGTTGGGC	69
	CHTR-P2	:	GGTGAAGTGCTTGCATGGATCT	70
10	LIS-P1	:	ACCTGTGAGTTTTCGTTCTTCTC	71
	LIS-P2	:	CTATTTGTTCAGTTTTGAGAGGTT	72
	LIS-P3	:	ATTTTCCGTATCAGCGATGATAC	73
	LIS-P4	:	ACGAAGTAAAGGTTGTTTTTCT	74
	LIS-P5	:	GAGAGGTTACTCTCTTTATGTCAG	75
15	LIS-P6	:	CTTTTATGTCAGATAAAGTATGCAA	202
	LIS-P7	:	CGTAAAAGGGTATGATTATTTG	203
	BRU-P1	:	TCGAGAATTGGAAAGAGGTC	204
	BRU-P2	:	AAGAGGTCGGATTTATCCG	205
	BRU-P3	:	TTCGACTGCAAATGCTCG	206
20	BRU-P4	:	TCTTAAAGCCGCATTATGC	207
	YEC-P1	:	CCTAATGATATTGATTCGCG	208
	YEC-P2	:	ATGACAGGTTAATCCTTACCCC	209

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EXAMPLE 1 : Pseudomonas aeruginosa

Pseudomonas aeruginosa is a significant human pathogen, usually in the context of serious underlying disease. It is also a major cause of nosocomial infections, which are characteristically prone to resistance to antimicrobial agents. This gram-negative, non-
5   fermentative rod can be responsible for different clinical manifestations, like wound infections, bacteremia, respiratory and urinary tract infections, and is also a major cause of morbidity and mortality in patients with cystic fibrosis.

Pseudomonas species are currently differentiated based on growth characteristics and several biochemical features implying a time schedule of 24h to 72h to get a correct
10   identification of the pathogen.

Already the development of monoclonal or polyclonal antibodies significantly improved the identification of Pseudomonas species. Recently however it has been shown that it is possible to detect organisms directly in clinical samples on a very sensitive and specific way using DNA probes with or without a prior amplification of the target DNA.

15   DNA probes to study Pseudomonas aeruginosa are already described and are mainly used for epidemiological typing (Ogle et al., 1987; Samadpour et al., 1988; McIntosh et al., 1992). However, none of these probes have been derived from the 16S-23S spacer.

The 16S-23S rRNA gene spacer region and a part of the 23S rRNA gene was amplified with conserved primers (upper primer: TGGGGTGAAGTCGTAACAAGGTA .
20   SEQ ID NO 155; lower primer: CCTTTCCCTCACGGTACTGGT, SEQ ID NO 156) using the polymerase chain reaction for the following species :

   - Pseudomonas aeruginosa 5669 ·
   - Pseudomonas alcaligenes LMG 1224^T
   - Pseudomonas fluorescens LMG 5167
25   - Pseudomonas putida LMG 2232
   - Pseudomonas stutzeri LMG 2333^T
   - Pseudomonas pseudoalcaligenes LMG 1225^T

To facilitate cloning of the obtained amplicons a NotI recognition site was added to the lower primer. After purification and digestion of the fragment with NotI, the amplicon
30   was cloned in a EcoRV/NotI digested pBluescript SK$^+$ plasmid vector.

Sequencing of the 16S-23S rRNA gene spacer region was performed according the

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dideoxy-chain terminating chemistry either using double stranded plasmid DNA combined with primers located in the plasmid vector or directly on the PCR products after purification combined with internal PCR primers.

Fig. 36 to 40 represent the nucleotide sequence of the 16S-23S rRNA gene spacer regions from the different Pseudomonas species described above. For P. fluorescens only partial sequence information was obtained.

From the nucleic acid sequence of the spacer from P. aeruginosa strain 5669 five oligonucleotide-probes were chosen and chemically synthetized. The sequences of the oligonucleotides are the following :

PA1 = PA-ICG 1 : TGGTGTGCTGCGTGATCCGATA

PA2 = PA-ICG 2 : TGAATGTTCGTGGATGAACATTGATT

PA3 = PA-ICG 3 : CACTGGTGATCATTCAAGTCAAG

Specificity and sensitivity testing of the oligonucleotide-probes was carried out using a reverse hybridization assay. Genomic DNA of the different bacteria tested was amplified using biotinylated primers (idem primers as for cloning procedure, see above). The obtained amplicon, spanning the 16S-23S rRNA gene spacer region, was denatured and hybridized to a membrane-strip onto which the different oligonucleotide probes were immobilized in a line-wise fashion (LiPA). Hybridization was carried out in a mixture of 3xSSC (1xSSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and 20% formamide (FA) at a temperature of 50° C for one hour. Washing was done in the same mixture at the same temperature for 15 min.

Hybrids were detected using a streptavidine conjugate coupled to alkaline phosphatase and the probes were visualized through a precipitation reaction using NBT (nitrobluetetrazolium) and BCIP (bromo-chloro-indolylphosphate).

The hybridization results obtained with probes PA1, PA2 and PA3 are given in table 4 and show that probes PA1 and PA3 were 100% specific for Pseudomonas aeruginosa and hybridized to all the strains tested. The hybridization signal with probe PA3 at 50° C was not optimal, so the oligonucleotide-probe was improved by adding some additional nucleotides to the specific probe. This newly designed probe is PA5.

PA5 = PA-ICG 5 : CTCTTTCACTGGTGATCATTCAAGTCAAG

Hybridization experiments with probe PA5 proved that this probe also shows a 100% specificity and 100% sensitivity for P. aeruginosa.

Oligonucleotide-probe PA2 hybridized only to 5 out of 17 P. aeruginosa strains tested.

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Direct sequencing of the 16S-23S rRNA gene spacer region of the strains which did not hybridize to these probes, showed some heterogeneity between different strains. Two mismatches were seen in comparison to the first developed PA2 probe. To overcome this heterogeneity between different strains in the region of probe PA2 a new probe PA4 was designed. This probe is degenerated at the position of the mismatches and some additional nucleotides were added to improve the hybridization signal at 50° C.

PA4 = PA-ICG 4 : TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC

A 100% specificity and 100% sensitivity was obtained with this degenerated probe as is shown by the hybridization results.

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taxa tested	PA1	PA2	PA3	PA4	PA5
Pseudomonas aeruginosa	17/17	5/17	17/17	17/17	17/17
Pseudomonas alcaligenes	0/1	0/1	0/1	0/1	0/1
Pseudomonas fluorescens	0/1	0/1	0/1	0/1	0/1
Pseudomonas putida	0/1	0/1	0/1	0/1	0/1
Pseudomonas pseudoalcaligenes	0/1	0/1	0/1	0/1	0/1
Pseudomonas stutzeri	0/1	0/1	0/1	0/1	0/1
Pseudomonas cepacia	0/1	0/1	0/1	ND	ND
Neisseria gonorrhoeae	0/1	0/1	0/1	ND	ND
Escherichia coli	0/1	0/1	0/1	ND	ND
Bordetella pertussis	0/1	0/1	0/1	ND	ND
Bordetella parapertussis	0/1	0/1	0/1	ND	ND
Bordetella bronchiseptica	0/1	0/1	0/1	ND	ND
Mycobacterium tuberculosis	0/1	0/1	0/1	ND	ND
Mycobacterium avium	0/1	0/1	0/1	ND	ND
Moraxella catarrhalis	0/4	0/4	0/4	ND	ND
Haemophilus influenzae	0/2	0/2	0/2	ND	ND
Streptococcus pneumoniae	0/3	0/3	0/3	ND	ND
Acinetobacter calcoaceticus	0/1	0/1	0/1	ND	ND
Staphylococcus aureus	0/2	0/2	0/2	ND	ND

Table 2 : **Hybridization results for Pseudomonas**
(n/m: number of strains positive/number of strains tested)

(ND: not done)

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EXAMPLE 2: Mycobacterium

A variety of mycobacterial species may be involved in serious human infectious disease. Notorious examples are *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Recently other species such as *M. avium*, *M. intracellulare* and *M. kansasii* have been more frequently encountered as human pathogens especially in immunocompromised hosts.

Consequently, laboratory diagnosis of mycobacterial infections should not be restricted to the *M. tuberculosis* complex but should ideally include most other clinically relevant mycobacterial species.

The identification and differentiation of pathogenic mycobacteria at the species level by conventional laboratory techniques is, in general, difficult and time-consuming.

To overcome these problems DNA-techniques were implemented. The techniques described extended from straightforward DNA-probing to automated sequence analysis. Several approaches have been recently reported (Jonas et al., 1993; Frothingham and Wilson, 1993; Tomioka et al., 1993; Saito et al., 1989; Vaneechoutte et al., 1993; Telenti et al., 1993; Böddinghaus et al., 1990).

However, these methods all have their particular disadvantages, and most of them still rely on culture. Moreover, and most importantly, none of these techniques allows for a simultaneous detection of the different clinically relevant mycobacterial species in a single test run. Besides, the differentiation of particular groups within the *Mycobacterium avium-intracellulare* complex is problematic and often even impossible.

To overcome the above-mentioned disadvantages, a LiPA-test was developed which allows for the simultaneous and reliable detection and differentiation of a number of *Mycobacterium* species and groups. The sets of probes used to achieve these goals were all derived from the 16S-23S rRNA spacer region. The methods used are analogous to those mentioned in example 1.

The 16S-23S rRNA spacer region, and part of the 16S and 23S rRNA flanking genes, was amplified by PCR with primers conserved for the genus *Mycobacterium*. At least one of the following primers located in the 16S gene were used as upper primers:

MYC-P1: TCCCTTGTGGCCTGTGTG   (SEQ ID NO 65)

MYC-P5: CCTGGGTTTGACATGCACAG   (SEQ ID NO 192)

At least one of the following primers, located in the 23S gene, were used as lower primers

for the amplification:

MYC-P2:	TCCTTCATCGGCTCTCGA	(SEQ ID NO 66)
MYC-P3:	GATGCCAAGGCATCCACC	(SEQ ID NO 67)
MYC-P4:	CCTCCCACGTCCTTCATCG	(SEQ ID NO 68)

All the above mentioned primers amplified the spacer region of all *Mycobacterium* strains tested, except primer MYC-P2 which was not functional for *M. chelonae*. In order to enhance the sensitivity of the detection, a nested PCR was sometimes carried out, using P5 and P4 as outer primers and P1 and P3 as inner primers.

In order to be able to design and select the probes and probe combinations which fit our purpose, the 16S-23S rRNA spacer region of a number of mycobacterial strains was sequenced. The obtained sequences were compared to each other and to those already known from literature (e.g. Frothingham et al., 1993, 1994; Kempsell et al., 1992; Suzuki et al., 1988; EP-A-0395292; Van der Giessen et al., 1994;) or from publicly accessable data banks. The corresponding sequences are represented in fig.1 to 35 (SEQ ID NO 76 to SEQ ID NO 110).

The probes derived from these data were all adjusted in such a way that the desired hybridization-behaviour was obtained using unified hybridization and wash conditions (i.e. 3xSSC, 20% deionized formamide, 50°C). The set of adjusted probes used for hybridization to different mycobacterial strains is represented in table 1a, SEQ ID NO 1-33. Please note that the probe nomenclature used in this example is an abbreviated version of the one used in table 1a: i.e. the letters "ICG" have always been omitted. According to the specific hybridization pattern obtained, the strains tested could be assigned to one of the following species or species groups: *M. tuberculosis* complex, *M. avium*, *M. intracellulare* or *M. intracellulare* complex, *M. kansasii*, *M. chelonae* and *M. gordonae*. The strains tested which belong to each group are summarized in Table 4. All strains were obtained from the Institute of Tropical Medecine, Antwerp, Belgium. The different probe-patterns obtained for each group are illustrated in Table 3, and are discussed in more detail hereafter.

M. tuberculosis complex

The *M. tuberculosis* complex harbours all strains belonging to *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*. The probes Mtb1, Mtb2 and Mtb3 hybridize with DNA originating from all *M. tuberculosis* complex strains tested. None of the other strains tested hybridized with these probes at the conditions used.

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In addition, *M. tuberculosis* complex strains, as is the case with all other mycobacterial strains tested, hybridize with either the **myc1** or the **myc22** probe or both. The latter two probes are designed as general *Mycobacterium* probes, either alone or in combination with each other.

5   *M. avium/M. paratuberculosis*

All *M. avium* and *M. paratuberculosis* strains studied reveal an identical hybridization pattern with the set of probes. For this type of organisms positive hybridization signals are obtained with the probes **myc1/myc22, mai1, mil11, mav1, mah1** and **mav22**. The latter two probes hybridize exclusively with *M. avium* and *M.*

10   *paratuberculosis* strains, and can thus be used as species-specific probes. Since the 16S-23S spacer sequences of *M. avium* isolates and *M. paratuberculosis* isolates are identical or nearly identical these two taxa cannot be discriminated from each other. This finding supports 16S rRNA sequencing data which indicate that *M. avium* and *M. paratuberculosis* should in fact be considered as belonging to one geno-species

15   (Rogal et al., 1990), *M. avium* ssp. *avium* and *M. avium* ssp. *paratuberculosis*.

M. intracellulare and **M. intracellulare** complex (MIC)

MIC strains are genotypically highly related organisms, which, according to sequence data of the 16S-23S rRNA spacer region, belong to a distinct cluster which is separate from other *Mycobacterium* species. *M. avium* and *M. scrofulaceum* are their closest

20   relatives. Almost all strains tested which are generally referred to as *M. avium* complex (MAC) strains (the former MAIS-complex) can be found in the MIC group. Thus, the MIC group defined in the current invention encompasses the MAC-type strains described by Frothingham and Wilson (1993) with the exception of MAC-G which appears to be *M. scrofulaceum*. Also *M. intracellulare* strains *sensu stricto* (*M.*

25   *intracellulare s.s.*) are part of this cluster.

Because this MIC group contains a quite large group of strains with, among them, subgroups showing different hybridization characteristics to the set of probes, a further subdivision into MIC-types was envisaged.

Type MIC 1 harbours *M. intracellulare s.s.*, together with some other MAC-strains.

30   All MIC 1 type isolates, without exception, hybridize to the following probes: **myc1/myc22, mai1** and **mac1**. The following probes can be used to make further subdivisions within the MIC 1 group : **mil11, min1, min2 to 2222, mil22** and

mhef1.

M. intracellulare sensu stricto strains (type <u>MIC 1.1.a</u>) can be distinguished from other subtypes in this group by virtue of probe **min1** which is positive only for this group of strains. All strains of type MIC 1.1.a strains are positive when tested with the *M. intracellulare* probe of the Gen-Probe Rapid Diagnostic system for MAC.

Type <u>MIC 1.1.b</u> and <u>MIC 1.2</u> harbour strains which are highly related to *M. intracellulare*. They can be differentiated by using probes **mil11** and **mil22** (see Table 3). Further subdivision within these groups was not attempted although this could be achieved by using the probes : **min2, min22, min222** and **min2222**. Further subdivision might be of value for epidemiological reasons.

Only two of our collection of strains tested group as <u>MIC 2</u> strains. One of these strains is a "*Mycobacterium lufu*" strain (ITG 4755). The specific probe pattern generated by these strains is characterized by a positive hybridization signal with the following probes : **myc1/myc22, mai1, mil22, mah1** and **mal1**. Variable hybridization results are obtained with probes **min2222, mac1** and **mhef1**. The other probes are negative. It is not unlikely that MIC 2 would eventually prove to be a heterogeneous group when more strains of this type are being identified. The variable probes may help in a further differentiation, if this would become relevant.

Type <u>MIC 3</u> groups a fairly high number of MAC-strains which are rather remotely related to *M. intracellulare s.s.* strains and most other MAC-strains. This cluster should be regarded as distinct from *M. avium* and *M. intracellulare* on genotypical grounds. All MIC 3 subtypes hybridize to probes **myc1/myc22, mai1, mil22** and **mco1**. A positive signal with the latter probe (mco1) is characteristic for MIC 3 strains. Variable hybridization results are obtained with the following probes : **mac1, mhef1** and **mah1**. MIC 3 can be further subdivided into four subtypes by using three probes : **mth11, mth2** and **mef11**. Probe mth2 is specific for type <u>MIC 3.1</u> which encompasses a group of highly related MAC-strains isolated from immuno-compromised human beings. Most MIC 3 strains are located in the MIC 3.1 subtype. Eventually species status may be assigned to this group of strains, as might also be the case for other groups of MAC strains, yet unnamed. In subtypes <u>MIC 3.4</u>, <u>MIC 3.3</u> and <u>MIC 3.2</u> only two, one and one strain are found respectively in our collection of strains tested.

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Type <u>MIC 4</u> is a collection of "MAIS" strains (including *M. malmoense*) which are
remotely related to *M. intracellulare*. The only probe of the above-described set
which hybridizes to MIC 4, apart from the general myc1/myc22 probes, is the **mai1**
probe. This probe shows a broad specificity, hybridizing also with *M. avium*, *M.*
intracellulare and other MIC strains and *M. scrofulaceum*.

M. scrofulaceum

All *M. scrofulaceum* strains tested reveal an identical hybrdization pattern with the
set of probes. A positive signal with probe **msc1** is unique to *M. scrofulaceum*
strains. The only other probes with a positive signal for this species are evidently
myc1/myc22 and also mai1.

M. kansasii

Probes **mka3** and **mka4** are specific for *M. kansasii*; i.e. a distinct positive signal is
obtained on the LiPA strip when amplified DNA from the *M. kansasii* strains is used
in the hybridization whilst with all other organisms tested the signal is absent.
Although the sequences of probes **mka1** and **mka2** are not absolutely complementary
to the target sequence (3 and 1 mismatches, respectively), these probes also proved
to be useful since they hybridized exclusively to *M. kansasii* DNA and not to any
other mycobacterial DNA tested under the conditions used (50°C, 3xSSC, 20%
formamide). This illustrates that probes not necessarilly have to match perfectly to
the target to be useful, and that modifications in sequence and length may be allowed
up to a certain degree.

M. chelonae

The species *M. chelonae* encompasses *M. chelonae* ssp. *chelonae* and *M. chelonae*
ssp. *abscessus* strains. The spacer region was sequenced for one strain of each
subspecies and small differences were noticed (SEQ ID NO 103 and SEQ ID NO
102). Probes **mch1** and **mch2** hybridize to both strains. All other probes are negative
for these 2 strains except for myc1/myc22.

Upon testing of probes mch1 and mch2 with 2 additional *M. chelonae* strains not
mentioned in table 4, i.e. *M. chelonae* 94-379 and *M. chelonae* 94-330, both obtained
from the Institute of Tropical Medecine in Antwerp, Belgium, it appeared that they
did not hybridize to probe mch1. This was confirmed by sequencing the spacer region
of these two strains (SEQ ID N0 184). Cluster analysis of the spacer region with

other mycobacteria revealed that M. chelonae strains can be subdivided in two groups. A third probe **mch3** was designed to specifically detect this second group of strains, to which 94-379 and 94-330 belong.

This illustrates that the use of DNA probes derived from the 16S-23S rRNA spacer region can be helpful in differentiating different groups of strains, which belong to the same species according to the classical identification methods, and possibly can be used to detect and describe new species within the mycobacteria. In this case mch2 detects all *M. chelonae* strains, whereas mch1 and mch3 differentiate between different subgroups.

M. gordonae

The five *M. gordonae* strains tested all hybridize to probe **mgo5**. Positive hybridization signals are also obtained with probes myc1/myc22, and some *M. gordonae* strains also hybridize to probes mgo1 and mgo2.

other mycobacterial species

Strains belonging to other mycobacterial species than those mentioned above only hybridize to the general probes **myc1/myc22**. This indicates that these strains most probably belong to the genus *Mycobacterium*, but do not belong to one of the species or groups which can be specifically identified by using one or more of the other probes described.

In conclusion we can state that, according to the particular combinations of probes of the invention used, DNA probe tests at different levels can be provided.

When all probes are used in one and the same LiPA-test, differentiation at the species level as well as subtyping of certain groups of mycobacteria can be achieved. However, the probe-assembly on one strip could be restricted to those probes which are species-specific; in that case identification is performed at the species level. A further reduction of the number of probes on the strip might lead to the specific detection of only one or just a few species. Obviously, LiPA strips can be designed which solely attempt to subtype strains, e.g. those belonging to the *M. intracellulare* complex (MIC). Depending on the particular needs of the laboratoria performing diagnosis and/or typing of mycobacteria, all these different applications might be of value. However, it is clear that by using a combination of probes in a LiPA-format the amount of information obtained as to the identity of the organisms

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present in the clinical sample, is considerably increased as compared to DNA probe tests using only a single probe. For some groups, or at least for further subdivision of some groups, a single probe uniquely hybridizing to this (sub)group could not be designed. In that case only probe-patterns are able to provide the information needed. For these applications

5   the LiPA is an advantageous format.

Table 3 :   Different probe patterns obtained for mycobacterial (sub)species

Mycobacterium	myc1 myc22	mtb1 mtb2 mtb3	mai1	mil11	mav1 mav22	min1	min222	min22	min2	min2222	mil22	mac1
M. tuberculosis / M. bovis	+	+	-	-	-	-	-	-	-	-	-	-
M. avium / M. paratuberculosis	+	-	+	+	+	-	-	-	-	-	-	-
MIC 1.1.a	+	-	+	+	-	+	+	+	+	+	-	+
MIC 1.1.b	+	-	+	+	-	-	±	±	±	±	-	+
MIC 1.2	+	-	+	-	-	-	-	±	±	+	+	+
MIC 2	+	-	+	-	-	±	-	-	-	±	+	±
MIC 3.4	+	-	+	-	-	-	-	-	-	-	+	±
MIC 3.3	+	-	+	-	-	-	-	-	-	-	+	+
MIC 3.1	+	-	+	-	-	-	-	-	-	-	+	+
MIC 3.2	+	-	+	-	-	-	-	-	-	-	+	+
MIC 4	+	-	+	-	-	-	-	-	-	-	-	-
M. scrofulaceum	+	-	+	-	-	-	-	-	-	-	-	-
M. kansasii	+	-	-	-	-	-	-	-	-	-	+	-
M. chelonae	+	-	-	-	-	-	-	-	-	-	-	-
M. gordonae	+	-	-	-	-	-	-	-	-	-	-	-
Mycobacterium sp.	+	-	-	-	-	-	-	-	-	-	-	-

Table 3: continued

Mycobacterium	mco1	mth11	mth2	mef11	mhef1	mah1	mal1	msc1	mka1,2,3,4	mch 1,2,3	mgo1,2	mgo5
M. tuberculosis	-	-	-	-	-	-	-	-	-	-	-	-
M. bovis	-	-	-	-	-	-	-	-	-	-	-	-
M. avium	-	-	-	-	-	+	-	-	-	-	-	-
M. paratuberculosis	-	-	-	-	-	-	-	-	-	-	-	-
MIC 1.1.a	-	-	-	-	-	-	-	-	-	-	-	-
MIC 1.1.b	-	-	-	-	-	-	-	-	-	-	-	-
MIC 1.2	-	-	-	-	±	-	-	-	-	-	-	-
MIC 2	-	-	-	-	±	+	+	-	-	-	-	-
MIC 3.4	+	-	-	+	+	±	-	-	-	-	-	-
MIC 3.3	+	+	-	+	+	+	-	-	-	-	-	-
MIC 3.1	+	+	+	-	±	±	-	-	-	-	-	-
MIC 3.2	+	-	-	-	+	+	w	-	-	-	-	-
MIC 4	-	-	-	-	-	-	-	-	-	-	-	-
M. scrofulaceum	-	-	-	-	-	-	-	+	-	-	-	-
M. kansasii	-	-	-	-	-	-	-	-	+	-	-	-
M. chelonae	-	-	-	-	-	-	-	-	-	±	-	-
M. gordonae	-	-	-	-	-	-	-	-	-	-	±	+
Mycobacterium sp.	-	-	-	-	-	-	-	-	-	-	-	-

w : weak / v : very weak / ± : + or -, variable according to the strain tested

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Table 4   Mycobacteria strains tested in LiPA

species/group	strain numbers from Institute of Tropical Medecine Antwerp (except those between parentheses)
M. tuberculosis complex	7602, 8004, 8017, 8647, 8872, 9081, 9129, 9173, 9517, (ATCC 27294), 8324, 8428
M. avium/ M. paratuberculosis	1101, 1983, 2070, 2074, 4176, 4189, 4191, 4193, 4197, 4204, 4386, 4991, 5872, 5874, 5884, 5887, 5893, 5894, 5897, 5903, 5904, 5905, 5927, 5983, 8180, 8750, (ATCC 25291), M. paratub : (316F), (2E)
M. intracellulare (MIC 1.1.a)	4199, 4208, 5701, 5880, 5906, 5908, 5909, 5913, 5915, 5917, 5918, 5920, 5921, 5924, 5925, 5929, 8713, 8717, 8718, 8720, 8721, 8722, 8732, 8740, 8741, 8742, 8744, 8747, 8749
MIC 1.1.b	8694, 8745, 8754 8708 5513, 8743 8054, 8190
MIC 1.2	8710, 8711, 8712, 8714, 8715, 8716, 8725, 8729, 8733, 8737, 8746, 8751, 8752 5919 8695 8748
MIC 2	5922 4755 (M. lufu)
MIC 3.4	1815 8707
MIC 3.3	5620
MIC 3.1	925, 926, 1329, 1788, 1794, 1812, 1818, 2069, 2073, 2076, 4541, 4543, 5074, 5280, 5789, 7395, 8739, 8753 8738
MIC 3.2	5765
M. scrofulaceum	4979, 4988, 5907, 8706, 8726, 8727, 8735, (MB022), (MB023), (MB024)
M. kansasii	4987, (ATCC 22478)
M. chelonae	4975, 9855
M. gordonae	7703, 7704, 7836, 7838, 8059
MIC 4	8723, 8724 8757 4842 (M. malmoense)
other mycobacterial species	7732 (M. marinum), 94-123 (M. celatum), 778 (M. haemophilum), 8777 (M. genavense), 4484 (M. siniae), 4986 (M. xenopi), 4304 (M. fortuitum), 1837 (M. ulcerans)

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EXAMPLE 3: Listeria

Listeria species are a group of Gram-positive rods widely spread in nature. Within
this group it seems that only L. monocytogenes is pathogenic to humans and animals. L.

5   monocytogenes is the causative agent of listeriosis, giving rise to meningitis, abortions,
encephalitis and septicemia. Immunocompromised individuals, newborn infants and pregnant
women are high risk groups for this foodborn disease. Most cases have been caused by the
consumption of food of animal origin, particularly soft cheeses. Therefore, the presence of
L. monocytogenes should be excluded from food. For safety measurements, in some

10   countries, the absence of all Listeria species is required in food products.

The classical identification method for L. monocytogenes in dairy products involves
an enrichment culture for 48 h and subsequently colony forming on selective agar medium
for 48 h followed by a whole set of biochemical and morphological assays (Farber and
Peterkin, 1991). This procedure could be very much simplified by the use of gene probes.

15   Several DNA probes are already described for the identification of L. monocytogenes.
Some probes are derived from genes responsible for the pathogenicity of the organism, for
instance the listeriolysin O gene (Datta et al., 1993) or the invasion-associated-protein (iap)
(Bubert et al., 1992).

A commercially available identification system, based on a specific 16S rRNA probe,

20   was introduced by GenProbe (Herman and De Ridder, 1993; Ninet et al., 1992).

These specific probes are used as confirmation assays on colonies obtained after
enrichmnent and plating on selective agar medium.

Recently several publications reported on the use of the polymerase chain reaction to
amplify the target region for the DNA probes, which can shorten the time of the assay

25   without interfering with the specificity and the sensitivity of the assay. Different primer sets
are described that can specifically amplify L. monocytogenes DNA. These primer sets were
derived from the listeriolysin O gene (Golstein Thomas et al., 1991), and the iap gene (Jaton
et al., 1992).

We used the 16S-23S rRNA gene spacer region as the target for the development of

30   a genus-specific probe for Listeria and a probe specific for Listeria monocytogenes.

Using conserved primers derived from the 3' end of the 16S rRNA and the 5' end of
the 23S rRNA (sequences are given in example 1) the spacer region was amplified using the

polymerase chain reaction and subsequently cloned in a suitable plasmid vector following the same procedures as in example 3.

Two amplicons differing in length (800 bp and 1100 bp) were obtained. Both PCR fragments were cloned for the following Listeria species :

- Listeria monocytogenes, serovar 4b, IHE (Instituut voor Hygiëne en Epidemiologie, Belgium)

- Listeria ivanovii CIP 78.42 (Collection Nationale de Cultures de Microorganisms de l'Institut Pasteur, France)

- Listeria seeligeri serovar 4a, nr. 42.68 (Bacteriologisches Institut, Südd, Versuchs- und Forschungsanstalt für Milchwirtschaft Weihenstephan, Germany)

The sequence of the spacer region between the 16S and 23S rRNA gene was determined using the cloned material originating from the 800 bp PCR fragment and this was done for the three described Listeria species. Fig. 41 to 43 show the sequences of the different short spacer regions obtained. The sequence of this short spacer region of L. monocytogenes was also retrieved from the EMBL databank (LMRGSPCR).

Based on this sequence information, following oligonucleotides for species-specific detection were chosen and chemically synthesized :

LMO-ICG-1 : AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG

LMO-ICG-2 : TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC

LSE-ICG-1 : AGTTAGCATAAGTAGTGTAACTATTTATGACACAAG

LIV-ICG-1 : GTTAGCATAAATAGGTAACTATTTATGACACAAGTAAC

Also, a genus specific probe for Listeria was designed:

LIS-ICG-1 : CAAGTAACCGAGAATCATCTGAAAGTGAATC

The oligonucleotide-probes were immobilized on a membrane strip and following reverse hybridization with biotinylated PCR fragments, the hybrids were visualized using a precipitation reaction. The hybridization results of different Listeria species are summarized in table 5.

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Table 5

Species	n	LIS1	LMO1	LMO2	LSE1	LIV1
L. monocytogenes	1	+	+	+	−	−
L. seeligeri	2	+	+	±	+	±
L. ivanovii	3	+	±	−	±	+
L. welshimeri	3	+	+	±	−	−
L. innocua	2	+	+	+	−	−

These hybridization results show that probe LIS1 can detect all described Listeria species, but also that the species-specific probes cross-hybridize to each other. Hence, from this short spacer region probes with sufficient specificity could not be found.

For Listeria monocytogenes the 16S-23S rRNA gene spacer was also determined originating from the 1100 bp fragment. Fig. 45 shows the sequence obtained for this species. This sequence information was also obtained for L. seeligeri (see fig. 46) and partial sequence information of the large spacer region was obtained for L. ivanovii (see fig. 44).

Based on sequence alignment with L. seeligeri following oligonucleotide-probe was chosen to specifically detect L. monocytogenes.

LMO-ICG-3 : AGGCACTATGCTTGAAGCATCGC

Initial hybridization results (not shown) indicated that no cross-hybridization with other Listeria species was seen with this L. monocytogenes probe LMO3, and that all Listeria strains used hybridized to the general probe LIS1.

The oligonucleotide-probes, LIS1 for detection of all Listeria species and LMO3 for specific detection of L. monocytogenes, were immobilized on a membrane strip and hybridized to labeled amplicons, containing the 16S-23S rRNA spacer region, derived from different organisms. The hybridization results are shown in the following table.

An excellent specificity and sensitivity were obtained for probes LMO3 and LIS1 respectively at the species and genus level.

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Table 6

Taxa tested	n	LIS1	LMO3
Listeria monocytogenes	44	+	+
Listeria ivanovii	10	+	−
Listeria seeligeri	11	+	−
Listeria welshimeri	16	+	−
Listeria innocua	23	+	−
Listeria murrayi	3	+	−
Listeria grayi	2	+	−
Brochotrix thermosphacta	1	−	−
Brochotrix campestris	1	−	−
Bacillus cereus	3	−	−
Bacillus brevis	2	−	−
Bacillus coalgulans	1	−	−
Bacillus pumilis	1	−	−
Bacillus macerans	1	−	−
Bacillus lentus	1	−	−
Bacillus firmus	2	−	−
Bacillus subtilis	2	−	−
Bacillus megantum	1	−	−
Enterococcus faecalis	1	−	−
Enterococcus faecium	1	−	−
Enterococcus durans	1	−	−
Lactococcus lactis	3	−	−
Lactococcus caseï	1	−	−
Escherichia coli	1	−	−
Hafnia halvei	1	−	−
Agrobacterium tumefaciens	2	−	−
Mycoplasma dimorpha	1	−	−
Clostridium tyrobutyricum	1	−	−
Clostridium perfringens	1	−	−
Clostridium sporogenes	1	−	−
Clostridium acetobutyricum	1	−	−
Brucella abortus	1	−	−
Brucella suis	1	−	−
Brucella melitensis	1	−	−
Staphylococcus aureus	1	−	−
Salmonella typhimurium	1	−	−
Salmonella enteritidis	1	−	−
Yersinia enterocolitica	1	−	−

n: number of strains tested

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These two probes can be used for the detection of <u>Listeria</u> species and <u>Listeria</u> <u>monocytogenes</u> directly on food samples or after enrichment of the samples in liquid broth. In both cases amplification problems can occur with the conserved primerset due to the enormous background flora in these samples.

To circumvent this problem, we designed several sets of primers derived from the 16S-23S rRNA spacer regions of <u>Listeria</u> species.

Primers LIS-P1 and LIS-P2 are upper primers, whereas LIS-P3 and LIS-P4 are lower primers. These primersets amplify the smaller 16S-23S rRNA spacer region as well as the larger spacer of <u>Listeria</u> species (except <u>L. grayi</u> and <u>L. murrayi</u>). If needed these primers can be used in a nested PCR assay where LIS-P1/LIS-P4 are the outer primers and LIS-P2/LIS-P3 are the inner primers.

For the specific detection of <u>Listeria monocytogenes</u> probe LMO-ICG-3 was designed and derived from the large 16S-23S rRNA spacer region. In order to specifically amplify only this large spacer region for an improved detection of this pathogen directly in samples a set of primers was derived from the part of sequence information from the large 16S-23S rRNA spacer region that is not present in the smaller rRNA spacer. For this aim, primers LIS-P5 and LIS-P6 are used as the upper primers and LIS-P7 is used as the lower primer.

LIS-P1	: ACCTGTGAGTTTTCGTTCTTCTC	71
LIS-P2	: CTATTTGTTCAGTTTTGAGAGGTT	72
LIS-P3	: ATTTTCCGTATCAGCGATGATAC	73
LIS-P4	: ACGAAGTAAAGGTTGTTTTTCT	74
LIS-P5	: GAGAGGTTACTCTCTTTTATGTCAG	75
LIS-P6	: CTTTTATGTCAGATAAAGTATGCAA	202
LIS-P7	: CGTAAAAGGGTATGATTATTTG	203

During the evaluation of the probes for <u>Listeria</u> spp. an organism was isolated from cheese that resembled <u>Listeria</u> according to the classical determination methods. This isolate (MB 405) showed the following characteristics (similar to <u>Listeria</u> spp.) : Gram positive. growth on Oxford and Tryptic Soy Agar, catalase positive. The only difference with the <u>Listeria</u> spp. was the motility, which was negative.

Using the conserved primers as described in example 1 in order to amplify the 16S-23S rRNA spacer region of this isolate MB 405, the same amplicon pattern was obtained with this strain as with <u>Listeria</u> spp. Hybridization of the amplicon showed that there was no

signal obtained with any of the probes for <u>Listeria</u> spp.

Sequencing of the 16S rRNA of isolate MB 405 and subsequent comparison with <u>Listeria</u> spp. and relatives showed that the organism was more closely related to <u>Listeria</u> spp. than to any other species described in the literature until now. Taxonomical studies will show if this isolate does or does not belong to the genus <u>Listeria</u>. This isolate, and subsequently isolated organisms from the same type, are referred to in this application as <u>Listeria</u> like organisms.

Isolate MB 405 seemed to contain at least 3 different 16S-23S rRNA spacer regions which were cloned and sequenced. Following alignment with <u>Listeria</u> spp. an oligonucleotide-probe was chosen te specifically detect <u>Listeria</u>-like strains:

LISP-ICG-1 : CGTTTTCATAAGCGATCGCACGTT

Reverse hybridization reactions of this probe with the 16S-23S rRNA spacer regions of <u>Listeria</u> spp. showed that there was no cross-hybridization.

EXAMPLE 4: Chlamydia trachomatis

Chlamydia trachomatis is a small obligate intracellular gram-negative bacterium, which has 15 serovars (A-K, Ba, L1, L2, and L3) distinguished by the major outer membrane protein (MOMP) and contains a cryptic plasmid required for intracellular growth. The A-K and Ba serovars constitute the trachoma biovar, while the L1, L2, and L3 serovars constitute the LGV biovar.

Serovars A, B, Ba, and C are commonly associated with trachoma, the leading cause of preventable blindness worldwide. The D-K serovars are found mainly in sexually transmitted infections and are the major cause of cervicitis and pelvic inflammatory disease in women, and urethritis and epididymitis in men. Serovars L1, L2 and L3 are involved in lymphogranuloma venereum, a rare sexually transmitted disease.

Cell culture is regarded as the benchmark method for laboratory diagnosis, although specimen viability is difficult to maintain during transport and laboratory techniques are time-consuming and technically demanding. Therefore, a number of more rapid test kits were developed, such as an enzyme-linked immunosorbent assay, and direct fluorescent-antibody staining. However, none of these immunoassays have been shown to have high levels of sensitivity or specificity.

A nonisotopic DNA probe assay (Gen-Probe PACE; Woods et al., 1990) that detects chlamydial rRNA is commercially available. Recently, the polymerase chain reaction (PCR) method has been used for detection of Chlamydia infections. Detection was targeted at either the cryptic plasmid (Loeffelholz et al., 1992), or the omp1 gene, which encodes for the major outer membrane protein (Taylor-Robinson et al., 1992). Compared with other techniques, PCR has higher sensitivity and specificity (Ossewaarde et al., 1992). None of these assays make use of DNA probes derived from the 16S-23S rRNA gene spacer region.

For a Chlamydia trachomatis L2 and a Chlamydia psittaci 6BC strain, a part of the ribosomal RNA cistron, containing the 16S-23S rRNA spacer region was amplified using conserved primers (see example 1) and subsequently cloned in a plasmid vector. The 16S-23S rRNA spacer region was sequenced using the dideoxychain terminating chemistry.

The sequence of the spacer region of both Chlamydia species is shown in fig. 47 to 48.

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Based on this sequence information, following oligonucleotide-probes were chemically synthetized :

CHTR-ICG-1 : GGAAGAAGCCTGAGAAGGTTTCTGAC

CHTR-ICG-2 : GCATTTATATGTAAGAGCAAGCATTCTATTTCA

CHTR-ICG-3 : GAGTAGCGTGGTGAGGACGAGA

CHPS-ICG-1 : GGATAACTGTCTTAGGACGGTTTGAC

The oligonucleotide-probes were immobilized in a line-wise fashion on a membrane strip and subsequently used in a reverse hybridization assay with biotinylated PCR products, containing the 16S-23S rRNA spacer region, as target.

Hybridizations were done in a solution of 3xSSC and 20% formamide (FA) at a temperature of 50°C.

The hybridization results with the different probes are shown in the following table.

Table 7

Strains tested	CHTR1	CHTR2	CHTR3	CHPS1
Chlamydia trachomatis L2	+	+	+	—
Chlamydia psittaci 6BC	—	—	—	+
Chlamydia psittaci CP	—	—	—	+
Chlamydia psittaci TT	—	—	—	+
Haemophilus ducreyi CIP 542	—	—	—	—
Haemophilus influenzae NCTC 8143	—	—	—	—
Neisseria gonorrhoeae NCTC 8375	—	—	—	—
Moraxella catarrhalis LMG 5128	—	—	—	—
Escherichia coli B	—	—	—	—
Streptococcus pneumoniae S92-2102	—	—	—	—

As shown in the table at a hybridization temperature of 50°C the probes CHTR1, CHTR2 and CHTR3 are specific for Chlamydia trachomatis and probe CHPS1 is specific for Chlamydia psittaci.

Several clinical isolates, obtained from the SSDZ, Delft, Netherlands, identified as Chlamydia trachomatis using conventional methods were tested in a reverse hybridization assay with the different oligonucleotide-probes. All Chlamydia trachomatis specific probes gave a positive hybridization signal and none of the isolates reacted with the Chlamydia psittaci probe. For some clinical isolates the CHTR2 probe reacted significantly weaker than

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CHTR1 or CHTR3. The spacer region of one of these isolates (94 M 1961) was sequenced (SEQ ID NO 197) and the sequence revealed one mismatch with the spacer sequence of strain L2. An additional probe (CHTR4) was derived from this new spacer sequence :

CHTR-ICG-4 : GAGTAGCGCGGTGAGGACGAGA   (SEQ ID NO 201)

This probe gives a stronger hybridization signal than CHTR2 with some clinical isolates from Chlamydia trachomatis. It can be used alone, or in combination with the CHTR2 probe (e.g. both probes applied in one LiPA-line).

In order to develop very sensitive assays for the detection of Chlamydia trachomatis directly in clinical specimens a specific primerset was derived from the 16S-23S rRNA spacer region, CHTR-P1 (upper primer) and CHTR-P2 (lower primer), amplifying specifically the spacer region of Chlamydia species.

CHTR-P1   : AAGGTTTCTGACTAGGTTGGGC   69

CHTR-P2   : GGTGAAGTGCTTGCATGGATCT   70

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EXAMPLE 6: Mycoplasma pneumoniae and Mycoplasma genitalium

Mycoplasmas are a group of the smallest prokaryotes known that are able to grow in cell-free media, lack a cell wall, and have very small genomes with a low G+C content.
5 More than 100 different species have been isolated from humans, animals, plants, and insects.

In humans, mycoplasmas have been recognized either as pathogenic organisms or as commensals. The best known pathogen is Mycoplasma pneumoniae, the causative agent of primary atypical pneumonia, especially in children and young adults. The diagnosis of M.
10 pneumoniae has been based on the direct isolation by the culture method or on the detection of specific antibodies against M. pneumoniae in the patient's serum.

Another pathogen, first isolated from urethral specimens from patients with nongonococcal urethritis, has been described as Mycoplasma genitalium. This mycoplasma has several properties in common with M. pneumoniae. Both species are pathogenic, and
15 both possess the capability to adhere to erythrocytes, various tissue cells, glass, and plastic surfaces. Furthermore, M. genitalium and M. pneumoniae share antigens, giving rise to extensive cross-reactions in serological tests. The observation that M. genitalium could also be found in respiratory tract specimens from patients with pneumonia and isolated from a mixture with M. pneumoniae has raised questions to the possible pathogenicity of M.
20 genitalium.

Since cultivation of both species is time-consuming and serology lacks specificity, more rapid and more specific assays were developed to identify these mycoplasmas. The use of hybridization assays with DNA probes was described for these species, but despite good specificities these tests do not allow the detection of low levels of M. pneumoniae or M.
25 genitalium. So more recently, DNA hybridization techniques were developed using the polymerase chain reaction. M. pneumoniae-specific PCR assays have been reported using the P1 adhesin gene (Buck et al., 1992) and the 16S rRNA gene (Kuppeveld et al., 1992). Specific PCR assays for M. genitalium were described using sequences from the adhesin gene and the 16S rRNA gene.
30 The spacer sequences of clinical isolates of M. pneumoniae and M. genitalium (obtained from U. Göbel, University of Freiburg, Germany) were determined. They are shown in fig. 49 to 50. The sequences show some differences to those from other strains of

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the same species deposited in the EMBL databank (MPMAC and MGG37 respectively).
Based on this information four probes were derived: one general Mycoplasma probe, two M.
pneumoniae specific, and one M. genitalium specific probe :

Mycoplasma-ICG: CAAAACTGAAAACGACAATCTTTCTAGTTCC

5   MPN-ICG-1:   ATCGGTGGTAAATTAAACCCAAATCCCTGT

MPN-ICG-2:   CAGTTCTGAAAGAACATTTCCGCTTCTTTC

MGE-ICG-1:   CACCCATTAATTTTTTCGGTGTTAAAACCC

The probes were applied to LiPA strips and hybridized under standard conditions (3X
SSC, 20% formamide at 50°C) to amplified spacer material from four M. pneumoniae
10   strains, one M. genitalium strain and twenty-two non-Mycoplasma species strains. The
general probe hybridized only to the five Mycoplasma strains tested, while the specific
probes hybridized only to strains of the species for which they were designed.

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EXAMPLE 7: Other mycobacterial species

With the steady improvement of laboratory techniques the information on the systematics and clinical significance of the so called "potentially pathogenic environmental
5   mycobacteria" increased rapidly. With the emergence of newly recognized diseases, additional syndromes associated with different mycobacterial species have emerged and have assumed major importance.

In order to extend the LiPA test for the simultaneous detection of different mycobacterial species as described in example 2, a new set of DNA probes was designed to
10   specifically identify the following species : Mycobacterium ulcerans, Mycobacterium genavense, Mycobacterium xenopi, Mycobacterium simiae, Mycobacterium fortuitum, Mycobacterium malmoense, Mycobacterium celatum and Mycobacterium haemophilum.

These probes were derived from the 16S-23S rRNA spacer region sequence. For the above mentioned species this information was obtained through direct sequencing of PCR
15   products or after cloning of the PCR-amplified spacer region. The sequences obtained are represented in fig. 80 to 97, and in fig. 38 for M. malmoense.

The sequences of the spacer region of the above-mentioned mycobacterial species were compared and aligned to those already described in example 2 or in publicly available sources. From the regions of divergence, species-specific DNA probes were designed. The
20   probes were selected and designed in such a way that the desired hybridization behaviour (i.e. species-specific hybridization) was obtained under the same conditions as those specified for the other mycobacterial probes mentioned in example 2, i.e. 3X SSC, 20% deionized formamide, 50°C. This allows simultaneous detection of at least two, and possibly all, of the mycobacterial species described in the current invention.

25   The following oligonucleotide probes were designed from the spacer region sequence of respectively M. ulcerans, M. genavense, M. xenopi, M. simiae, M. fortuitum, M. malmoense, M. celatum and M. haemophilum:

MUL-ICG-1 : GGTTTCGGGATGTTGTCCCACC

MGV-ICG-1 : CGACTGAGGTCGACGTGGTGT

30   MGV-ICG-2 : GGTGTTTGAGCATTGAATAGTGGTTGC

MXE-ICG-1 : GTTGGGCAGCAGGCAGTAACC

MSI-ICG-1 : GCCGGCAACGGTTACGTGTTC

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MFO-ICG-1 : TCGTTGGATGGCCTCGCACCT

MFO-ICG-2 : ACTTGGCGTGGGATGCGGGAA

MML-ICG-1: CGGATCGATTGAGTGCTTGTCCC

MML-ICG-2: TCTAAATGAACGCACTGCCGATGG

MCE-ICG-1: TGAGGGAGCCCGTGCCTGTA

MHP-ICG-1: CATGTTGGGCTTGATCGGGTGC

The probes were immobilized on a LiPA strip and hybridized with amplified biotinylated material derived from a set of representative mycobacterial species as described in example 2. Amplification of the spacer region was carried out by PCR using a primer set as described in example 2. The different strains used for specificity testing are shown in table 8 together with the hybridization results obtained. The strains were obtained from the collection of the Institute for Tropical Medicine, Antwerp, Belgium.

The probes tested (MSI-ICG1, MXE-ICG-1, MFO-ICG-1, MFO-ICG-2, MML-ICG-1, MML-ICG-2, MCE-ICG-1 and MHP-ICG-1) specifically detected M. simiae, M. xenopi, M. fortuitum, M. malmoense, M. celatum and M. haemophilum respectively and showed no cross-hybridization with the other mycobacterial species tested. Thus, these probes allow a specific detection of mycobacterial species which were not further identifiable using the set of DNA probes described in example 2. M. malmoense was classified in example 2 as a "MIC 4"-type, while the other species mentioned above were only hybridizing to the general probes MYC1/MYC22 for the genus Mycobacterium, and were thus classified in example 2 as "other mycobacterial species".

All tested M. genavense isolates reacted with MGV-ICG1 and MGV-ICG2, and not with MSI-ICG1 designed for M. simiae, closely related to M. genavense. A group of "intermediate" organisms, situated in between M. simiae and M. genavense, were received from the Tropical Institute of Medecine, Antwerp, where they were classified as "M. simiae - like" (strains 4358, 4824, 4833, 4844, 4849, 4857, 4859, 7375, 7379, 7730, 9745, 94-1228). These strains reacted only with probe MGV-ICG2 and not with probe MSI-ICG1 which specifically detects M. simiae strains sensu stricto. Sequencing of the 16S-23S rRNA spacer region of two of these "M. simiae-like" isolates (strains 7379 and 9745) (see SEQ ID NO 161 and 162) confirmed that they were more closely related to M. genavense than to M. simiae. A new probe MGV-ICG3 was designed to specifically detect this group of organisms, which possibly belong to a new species.

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MGV-ICG 3 : TCGGGCCGCGTGTTCGTCAAA

This illustrates again that the use of DNA probes derived from the 16S-23S spacer region can be helpful in differentiating different groups of strains, which are also found indeterminate by classical taxonomic criteria. The use of these DNA probes may possilby lead to the description of new (sub)species within mycobacteria. In this case, the MGV-1 probe would react only with M. genavense strains sensu stricto, MGV-3 probe would react only with the intermediate "M. simiae-like" strains, and MGV-2 probe would detect both types of strains.

The probe MUL-ICG-1 reacted with all M. ulcerans strains tested, but also showed cross-hybridization with M. marinum strain ITG 7732. Sequencing of the spacer region of this M. marinum strain indeed revealed an identical sequence to that of M. ulcerans strain 1837 (see fig. 80). Further differentiation between M. marinum and M. ulcerans can be done using a probe from the 16S-rRNA gene of M. ulcerans, part of which is co-amplified with the spacer region when primers MYC P1-P5 are used for amplification. A species-specific 16S rRNA probe for M. ulcerans, which can work under the same hybridization conditions as the spacer probes for mycobacterium species differentiation, is for example:

TGGCCGGTGCAAAGGGCTG   (SEQ ID NO 216)

The above paragraph shows that, although it is preferable to use probes derived from the spacer region, it is also possible, and sometimes necessary, to combine the spacer probes with probes derived from other gene sequences, e.g. the 16S rRNA gene. Here again, these additional probes are selected such that they show the desired hybridization characteristics under the same hybridization and wash conditions as the spacer probes.

For M. kansasii, additional strains to the ones mentioned in example 2 have been tested with probes MKA-ICG-1, 2, 3 and 4 described in example 2. Since none of these probes was entirely satisfactory, additional probes were designed for M. kansasii detection. Therefor, the spacer region of some of the additional M. kansasii strains ITG 6328, 8698 and 8973 was sequenced (see fig.90 to 92). These strains were also obtained from the Institute of Tropical Medecine in Antwerp, Belgium. Apparently, M. kansasii strains constitute a quite heterogeneous group, with remarkable differences in the spacer sequence between different strains. Additional probes MKA-ICG-5, 6, 7, 8, 9 and 10 were designed, all hybridizing again under the same conditions as those earlier described, i.e. 3X SSC, 20% deionized formamide, 50°C. The probes were tested with a collection of test strains obtained from the

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Institute of Tropical Medicine, Antwerp, Belgium, and results are shown in table 8.

None of the M. kansasii probes hybridizes with a species other than M. kansasii, as far as tested. However, due to the heterogeneous character of this species, none of the M. kansasii probes hybridizes with all M. kansasii strains. The different M. kansasii probes recognize different strains of M. kansasii. This differential hybridization may be of clinical significance. On the other hand, if detection of all M. kansasii strains is desirable, a combination of different M. kansasii probes can be envisaged.

Table 8: additional mycobacterial probes

species/type	strain	MUL ICG-1	MGV ICG-1	MGV 2	MGV 3	MXE ICG-1	MFO ICG-1	MFO ICG-2	MSI ICG-1	MML ICG-1	MML ICG-2	MCE ICG-1	MHP ICG-1
M. tuberculosis	8004	-	-	-		-	-		-	-		-	-
M. avium	5887	-	-	-		-	-		-	-		-	-
M. intracellulare	5915 5913	-	-	-		-	-		-	-		-	-
MIC 3.1 strain	1812	-	-	-		-	-		-	-		-	-
MIC-4 strain	8724		-	-						-			
M. scrophulaceum	4979	-	-	-		-	-		-	-		-	-
M. kansasii	4987 2795 6238 6362 8698 8973 8974 8971	-	- - - -	- -	-	- - -	- -	-	- - - -	- -		- -	- -
M. ulcerans	1837 3129 5114 5115	+ + + +	- - -	- - -	-	- - -	- -		- - -	- - -		- -	- -
M. marinum	7732	+	-	-		-	-		-	-		-	-
M. malmoense	4832 4842	- -	-	-		-	-		-	+ +		-	-
M. gordonae	7703	-	-			-	-		-	-		-	-

Table 8 continued

M. chelonae	4975	-	-	-	-	-	-	-	-		
	9855	-	-	-	-	-	-	-	-		
	94-330	-	-	-	-	-	-	-	-		
	94-379	-	-	-	-	-	-	-	-		
M. gordonae	94-123	-	-	-	-	-	-	-	+	-	
M. haemophilum	778	-	+	+	-	-	-	-	-	-	-
	3071	-	+	+	-	-	-	+	-	-	+
M. genavense and M. simiae-like	8777	-	+	+	-	-	-	-		
	9745	-	+	+	+	-	-	-		
	92-742	-	+	+	-	-	-	-		
	7379	-	+	+	+	-	-	-		
	9500	-	+	+	-	-	-	-		
M. simiae	4484	-	-	-	-	-	+	-		
	4485	-	-	-	-	-	+	-		
M. xenopi	4986	-	-	-	-	+	-	-		
M. fortuitum	4304	-	-	-	-	-	-	+		

- = negative reaction, + = positive reaction, w = weak reaction, ± = variable reaction, blanc = non tested

Table 8 continued

species/type	strain	MKA ICG-3	MKA ICG-4	MKA ICG-5	MKA ICG-6	MKA ICG-7	MKA ICG-8	MKA ICG-9	MKA-ICG-10
M. tuberculosis	8004	-	-	-	-	-	-	-	-
M. avium	5887	-	-	-	-	-	-	-	-
M. intracellulare	5915	-	-		-	-	-	-	-
	5913								
MIC 3.1 strain	1812	-	-		-	-	-		
MIC-4 strain	8724	-	-	-	-	-	-		
M. scrophulaceum	4979	-	-	-	-	-	-	-	-
M. kansasii	4987	+	+	-	-	-	-	-	+
	2795	+	+	-	-	-	-	-	+
	6238	+	-	+	-	-	+	+	+
	6362	+	-	+	-	-	+	+	+
	8698	-	-	-	-	+	-	+	w
	8973	-	-	-	+	-	+	-	-
	8974	-	-	-	+	-	+	-	-
	8971	-	-	-	+	-	+	-	-
M. ulcerans	1837		-	-	-	-	-	-	-
	3129		-	-	-	-	-	-	-
	5114				-				
	5115				-				
M. marinum	7732	-	-	-	-	-	-	-	-
M. malmoense	4832	-	-	-	-	-	-	-	-
	4842								
M. gordonae	7703	-	-	-	-	-	-	-	-

Table 8 continued

	Strain numbers
M. chelonae	4975 9855 94-330 94-379
M. celatum	94-123
M. haemophilum	778 3071
M. genavense and M. simiae-like	8777 9745 92-742 7379 9500
M. simiae	4484 4485
M. xenopi	4986
M. fortuitum	4304

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EXAMPLE 8: Brucella

Brucellosis is a very widespread and economically important zoonosis which also affects humans.

For the identification of Brucella spp., mainly bacteriological and immunological detection techniques are being used. These tests are time-consuming and often give false-positive results. Quick and reliable identification methods are being developed, mainly based on DNA amplification and hybridization.

Specific detection of Brucella spp. based on the amplification of a 43 kDa outer membrane protein (Fekete A. et al., 1990) or of a part of the 16S rRNA gene (Herman and De Ridder, 1992) were already described.

In order to develop specific DNA probes and primers for the detection of Brucella spp. we analyzed the 16S-23S rRNA gene spacer region. Using conserved primers (sequences are given in example 1) the spacer region was amplified and subsequently cloned into the Bluescript SK+ vector following the same procedures as in example 1.

The obtained amplicon of about 1400 bp in length was cloned for the following Brucella species :   - Brucella abortus NIDO Tulya biovar 3 (SEQ ID NO 154)

               - Brucella melitensis NIDO biovar 1 (SEQ ID NO 131)

               - Brucella suis NIDO biovar 1 (SEQ ID NO 132)

HindIII digestion of the constructs, followed by subcloning of the obtained fragments (n=3) facilitated the sequencing of the spacer region for the three described Brucella spp..

Fig. 56, 57 and 79 represent the sequences of the spacer regions obtained for the above-mentioned strains of respectively Brucella melitensis, Brucella suis and Brucella abortus.

Due to the high homology of these spacer region sequences between different Brucella species, no species-specific DNA probes were deduced from this sequence information, and only genus-specific probes were designed.

For this purpose, the following probes were chemically synthesized:

        BRU-ICG 1 : CGTGCCGCCTTCGTTTCTCTTT

        BRU-ICG 2 : TTCGCTTCGGGGTGGATCTGTG

        BRU-ICG 3 : GCGTAGTAGCGTTTGCGTCGG

        BRU-ICG 4 : CGCAAGAAGCTTGCTCAAGCC

The oligonucleotides were immobilized on a membrane strip and following reverse

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hybridization with biotinylated PCR fragments, the hybrids were visualized using a precipitation reaction. The hybridization results of the immobilized probes with different Brucella spp. and related organisms are represented in the table 9.

These hybridization results show that probes BRU-ICG 2, BRU-ICG 3 and BRU-ICG 4 are specific for Brucella spp. and can be used in a reverse hybridization assay for detection of these pathogens. Probe BRU-ICG 1 cross-hybridizes with Ochrobactrum antropi and Rhizobium loti strains, which are two taxonomically highly related organisms, but which are not expected to be present in the same sample material as used for Brucella detection.

As described in previous examples (e.g. 3 and 4) also for Brucella specific primers were chosen from the 16S-23S rRNA spacer region, in order to specifically amplify the spacer region from Brucella strains.

BRU-P1 and BRU-P2 are used as upper primers, while BRU-P3 and BRU-P4 are used as lower primers. When used in a nested PCR assay the combination BRU-P1/BRU-4 is the outer primerset whereas the combination BRU-P2/BRU-P3 is the inner primerset.

BRU-P1 :	TCGAGAATTGGAAAGAGGTC	204
BRU-P2 :	AAGAGGTCGGATTTATCCG	205
BRU-P3 :	TTCGACTGCAAATGCTCG	206
BRU-P4 :	TCTTAAAGCCGCATTATGC	207

TABLE 9

TAXA TESTED	n	BRU-ICG 1	BRU-ICG 2	BRU-ICG 3	BRU-ICG 4
Brucella abortus	6	+	+	+	+
Brucella suis	3	+	+	+	+
Brucella melitensis	4	+	+	+	+
Brucella ovis	2	+	+	+	+
Brucella canis	2	+	+	+	+
Brucella neotomae	1	+	+	+	+
Phyllobacterium rubiacearium	1	—	—	NT	NT
Ochrobactrum anthropi	8	+	—	—	—
Agrobacterium tumefaciens	2	—	—	NT	NT
Agrobacterium rhizogenes	1	—	—	NT	NT
Mycoplana dimorpha	1	—	—	NT	NT
Rhizobium loti	1	+	—	-	-
Rhizobium meliloti	1	—	—	NT	NT
Rhizobium leguminosarum	1	—	—	NT	NT
Bradyrhizobium japonicum	1	—	—	NT	NT
Brochothrix thermosphacta	1	—	—	NT	NT
Brochothrix campestris	1	—	—	NT	NT
Bacillus cereus	3	—	—	NT	NT
Bacillus brevis	2	—	—	NT	NT
Bacillus coalgulans	1	—	—	NT	NT
Bacillus pumilis	1	—	—	NT	NT
Bacillus macerans	1	—	—	NT	NT
Bacillus lentus	1	—	—	NT	NT
Bacillus firmus	2	—	—	NT	NT
Bacillus subtilis	2	—	—	NT	NT
Bacillus megantum	1	—	—	NT	NT
Enterococcus faecalis	1	—	—	NT	NT
Enterococcus faecium	1	—	—	NT	NT
Enterococcus durans	1	—	—	NT	NT
Lactobacillus lactis	3	—	—	NT	NT
Lactobacillus casei	1	—	—	NT	NT
Leuconostoc lactis	1	—	—	NT	NT
Escherichia coli	1	—	—	NT	NT
Hafnia halvei	1	—	—	NT	NT
Clostridium tyrobutyricum	1	—	—	NT	NT
Clostridium perfringens	1	—	—	NT	NT
Clostridium sporogenes	1	—	—	NT	NT
Clostridium acetobutyricum	1	—	—	NT	NT
Staphylococcus aureus	1	—	—	NT	NT
Salmonella enteritidis	1	—	—	NT	NT
Yersinia enterocolitica	1	—	—	NT	NT
Listeria monocytogenes	1	—	—	NT	NT
Listeria ivanovii	1	—	—	NT	NT
Listeria seeligeri	1	—	—	NT	NT
Listeria welshimeri	1	—	—	NT	NT
Listeria innocua	1	—	—	NT	NT
Listeria murrayi	1	—	—	NT	NT
Listeria grayi	1	—	—	NT	NT

NT = Not tested   n = number of strains tested

EXAMPLE 9: Staphylococcus aureus

Staphylococcus aureus is the staphylococcal species most commonly associated with human and animal infections. *Staphylococcus aureus* strains have been identified as important etiologic agents in both community-acquired and nosocomial infections. Recently nosocomial infection with methicillin-resistant *S. aureus* (MRSA) appear to be increasingly prevalent in many countries. The strains belonging to this species are also causative agents of food spoilage and poisoning.

In order to discriminate in a fast and specific way *S. aureus* strains from other staphylococci, the use of molecular techniques based on DNA probes and/or PCR were already described in the literature. Examples of target genes used for the development of these DNA based assays are the 16S rRNA gene (De Buyser at al., 1992; Geha et al, 1994), the *mec*A gene (Ubukata et al., 1992; Shimaoka et al., 1994) and the *nuc* gene (Brakstad et al., 1992; Chesneau et al., 1993).

As a target for the development of specific DNA probes we chose the 16S-23S rRNA gene spacer region. Amplification using conserved primers derived from the 16S and the 23S rRNA genes (sequences, see example 1) showed that the pattern obtained was not similar in all *S. aureus* strains tested. A lot of variation was seen in either the number of fragments obtained and in the size of these different fragments.

One spacer region from strain UZG 5728 and four spacer regions (differing in length) from strain UZG 6289 were cloned into Bluescript SK+ vector and subsequently sequenced. The sequences are represented in fig. 64 to fig. 68 (SEQ ID NO 139 to SEQ ID NO 143). For the development of specific DNA probes these different spacer regions were compared to each other and to the spacer region derived from *Staphylococcus epidermidis* strain UZG CNS41 (SEQ ID NO 144).

The following probes were chemically synthesized :

      STAU-ICG 1 : TACCAAGCAAAACCGAGTGAATAAAGAGTT
      STAU-ICG 2 : CAGAAGATGCGGAATAACGTGAC
      STAU-ICG 3 : AACGAAGCCGTATGTGAGCATTTGAC
      STAU-ICG 4 : GAACGTAACTTCATGTTAACGTTTGACTTAT

The oligonucleotides were immobilized on a membrane strip and following reverse hybridization with biotinylated PCR fragments, the hybrids were visualized using a

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colorimetric precipitation reaction.

The hybridization results of the immobilized probes with different *Staphylococcus* spp. and non-staphylococcal organisms are represented in Table 10.

These hybridization results show that only probes STAU-ICG 3 and STAU-ICG 4 are specific for *Staphylococcus aureus* strains. Probe STAU-ICG 1 reacts with all *Staphylococcus* spp. tested and probe STAU-ICG 2 cross-hybridizes with the *S. lugdinensis* strain. Neither probe STAU-ICG 3 nor probe STAU-ICG 4 detects all *S. aureus* strains tested, but when both probes are used simultaneously in a LiPA assay, all *S. aureus* strains tested hybridize with one of these probes or with both.

Table 10

Strains tested	n	STAU-ICG 1	STAU-ICG 2	STAU-ICG 3	STAU-ICG 4
Staphylococcus aureus	13	+	+	+	+
Staphylococcus aureus	10	+	+	-	+
Staphylococcus aureus	3	+	+	w	+
staphylococcus aureus	1	+	-	+	-
Staphylococcus epidermidis	11	+	-	-	-
Staphylococcus saprophyticus	1	+	-	-	-
Staphylococcus haemolyticus	1	+	-	-	-
Staphylococcus capitis	1	+	+	-	-
Staphylococcus lugdinensis	1	+	-	-	-
Staphylococcus hominis	1	+	-	-	-
Bordetella pertussis	1	-	-	-	-
Bordetella parapertussis	1	-	-	-	-
Bordetella bronchiseptica	1	-	-	-	-
Mycobacterium tuberculosis	1	-	-	-	-
Mycobacterium avium	1	-	-	-	-
Moraxella catarrhalis	4	-	-	-	-
Haemophilus influenzae	2	-	-	-	-
Streptococcus pneumoniae	3	-	-	-	-
Pseudomonas cepacia	1	-	-	-	-
Pseudomonas aeruginosa	3	-	-	-	-
Acinetobacter calcoaceticus	1	-	-	-	-

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CLAIMS

1. Method for the detection and identification of at least one micro-organism, or for the simultaneous detection of several micro-organisms in a sample, comprising the steps of:

(i)   if need be releasing, isolating and/or concentrating the polynucleic acids from the micro-organism(s) to be detected in the sample;

(ii)   if need be amplifying the 16S-23S rRNA spacer region, or a part of it, from the micro-organism(s) to be detected, with at least one suitable primer pair;

(iii)   hybridizing the polynucleic acids of step (i) or (ii) with a set of probes comprising at least two probes under the same hybridization and wash conditions, with said probes being selected from the sequences of table 1a or equivalents thereof, and/or from taxon-specific probes derived from any of the spacer sequences as represented in figures 1-103, with said taxon-specific probe being selected such that it is capable of hybridizing under the same hybridization and wash conditions as at least one of the probes of table 1a ;

(iv)   detecting the hybrids formed in step (iii);

(v)   identification of the micro-organism(s) present in the sample from the differential hybridization signals obtained in step (iv).

2. Method according to claim 1, wherein said sample is originating from the respiratory tract and wherein wherein the set of probes as defined in step (iii) comprises at least one probe chosen from the following spacer probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)
MAI-ICG-1 :	CAACAGCAAATGATTGCCAGACACAC	(SEQ ID NO 6)
MIL-ICG-11 :	GAGGGGTTCCCGTCTGTAGTG	(SEQ ID NO 7)
MIL-ICG-22 :	TGAGGGGTTCTCGTCTGTAGTG	(SEQ ID NO 8)
MAC-ICG-1 :	CACTCGGTCGATCCGTGTGGA	(SEQ ID NO 9)
MAV-ICG-1 :	TCGGTCCGTCCGTGTGGAGTC	(SEQ ID NO 10)

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MAV-ICG-22 :	GTGGCCGGCGTTCATCGAAA	(SEQ ID NO 11)
MIN-ICG-1 :	GCATAGTCCTTAGGGCTGATGCGTT	(SEQ ID NO 12)
MIN-ICG-2 :	GCTGATGCGTTCGTCGAAATGTGTA	(SEQ ID NO 13)
MIN-ICG-22 :	CTGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 14)
MIN-ICG-222 :	TGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 15)
MIN-ICG-2222 :	GGCTGATGCGTTCGTCGAAATGTGTAA	(SEQ ID NO 16)
MAL-ICG-1 :	ACTAGATGAACGCGTAGTCCTTGT	(SEQ ID NO 17)
MHEF-ICG-1 :	TGGACGAAAACCGGGTGCACAA	(SEQ ID NO 18)
MAH-ICG-1 :	GTGTAATTTCTTTTTAACTCTTGTGTGTAAGTAAGTG	
		(SEQ ID NO 19)
MCO-ICG-11 :	TGGCCGGCGTGTTCATCGAAA	(SEQ ID NO 20)
MTH-ICG-11 :	GCACTTCAATTGGTGAAGTGCGAGCC	(SEQ ID NO 21)
MTH-ICG-2 :	GCGTGGTCTTCATGGCCGG	(SEQ ID NO 22)
MEF-ICG-11 :	ACGCGTGGTCCTTCGTGG	(SEQ ID NO 23)
MSC-ICG-1 :	TCGGCTCGTTCTGAGTGGTGTC	(SEQ ID NO 24)
MKA-ICG-1 :	GATGCGTTTGCTACGGGTAGCGT	(SEQ ID NO 25)
MKA-ICG-2 :	GATGCGTTGCCTACGGGTAGCGT	(SEQ ID NO 26)
MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTCGAGAGTTGTC	(SEQ ID NO 28)
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)
MCH-ICG-3 :	GGTGTGGTCCTTGACTTATGGATAG	(SEQ ID NO 210)
MGO-ICG-1 :	AACACCCTCGGGTGCTGTCC	(SEQ ID NO 31)
MGO-ICG-2 :	GTATGCGTTGTCGTTCGCGGC	(SEQ ID NO 32)
MGO-ICG-5 :	CGTGAGGGGTCATCGTCTGTAG	(SEQ ID NO 33)
MUL-ICG-1 :	GGTTTCGGGATGTTGTCCCACC	(SEQ ID NO 175)

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MGV-ICG-1 :	CGACTGAGGTCGACGTGGTGT	(SEQ ID NO 176)
MGV-ICG-2 :	GGTGTTTGAGCATTGAATAGTGGTTGC	(SEQ ID NO 177)
MGV-ICG-3 :	TCGGGCCGCGTGTTCGTCAAA	(SEQ ID NO 211)
MXE-ICG-1 :	GTTGGGCAGCAGGCAGTAACC	(SEQ ID NO 178)
MSI-ICG-1 :	CCGGCAACGGTTACGTGTTC	(SEQ ID NO 179)
MFO-ICG-1 :	TCGTTGGATGGCCTCGCACCT	(SEQ ID NO 180)
MFO-ICG-2 :	ACTTGGCGTGGGATGCGGGAA	(SEQ ID NO 181)
MML-ICG-1 :	CGGATCGATTGAGTGCTTGTCCC	(SEQ ID NO 188)
MML-ICG-2 :	TCTAAATGAACGCACTGCCGATGG	(SEQ ID NO 189)
MCE-ICG-1 :	TGAGGGAGCCCGTGCCTGTA	(SEQ ID NO 190)
MHP-ICG-1 :	CATGTTGGGCTTGATCGGGTGC	(SEQ ID NO 191)
PA-ICG 1 :	TGGTGTGCTGCGTGATCCGAT	(SEQ ID NO 34)
PA-ICG 2 :	TGAATGTTCGTGGATGAACATTGATT	(SEQ ID NO 35)
PA-ICG 3 :	CACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 36)
PA-ICG 4 :	TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	
		(SEQ ID NO 37)
PA-ICG 5 :	CTCTTTCACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 38)
MPN-ICG 1 :	ATCGGTGGTAAATTAAACCCAAATCCCTGT	(SEQ ID NO 49)
MPN-ICG 2 :	CAGTTCTGAAAGAACATTTCCGCTTCTTTC	(SEQ ID NO 50)
MGE-ICG 1 :	CACCCATTAATTTTTTCGGTGTTAAAACCC	(SEQ ID NO 51)
Mycoplasma-ICG :	CAAAACTGAAAACGACAATCTTTCTAGTTCC	(SEQ ID NO 52)
STAU-ICG 1 :	TACCAAGCAAAACCGAGTGAATAAAGAGTT	(SEQ ID NO 53)
STAU-ICG 2 :	CAGAAGATGCGGAATAACGTGAC	(SEQ ID NO 54)
STAU-ICG 3 :	AACGAAGCCGTATGTGAGCATTTGAC	(SEQ ID NO 55)
STAU-ICG 4 :	GAACGTAACTTCATGTTAACGTTTGACTTAT	(SEQ ID NO 56)
ACI-ICG 1 :	GCTTAAGTGCACAGTGCTCTAAACTGA	(SEQ ID NO 57)
ACI-ICG 2 :	CACGGTAATTAGTGTGATCTGACGAAG	(SEQ ID NO 58)

and more preferably from the following spacer probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)

MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)
MAI-ICG-1 :	CAACAGCAAATGATTGCCAGACACAC	(SEQ ID NO 6)
MIL-ICG-11 :	GAGGGGTTCCCGTCTGTAGTG	(SEQ ID NO 7)
MIL-ICG-22 :	TGAGGGGTTCTCGTCTGTAGTG	(SEQ ID NO 8)
MAC-ICG-1 :	CACTCGGTCGATCCGTGTGGA	(SEQ ID NO 9)
MAV-ICG-1 :	TCGGTCCGTCCGTGTGGAGTC	(SEQ ID NO 10)
MAV-ICG-22 :	GTGGCCGGCGTTCATCGAAA	(SEQ ID NO 11)
MIN-ICG-1 :	GCATAGTCCTTAGGGCTGATGCGTT	(SEQ ID NO 12)
MAL-ICG-1 :	ACTAGATGAACGCGTAGTCCTTGT	(SEQ ID NO 17)
MCO-ICG-11 :	TGGCCGGCGTGTTCATCGAAA	(SEQ ID NO 20)
MTH-ICG-11 :	GCACTTCAATTGGTGAAGTGCGAGCC	(SEQ ID NO 21)
MTH-ICG-2 :	GCGTGGTCTTCATGGCCGG	(SEQ ID NO 22)
MEF-ICG-11 :	ACGCGTGGTCCTTCGTGG	(SEQ ID NO 23)
MSC-ICG-1 :	TCGGCTCGTTCTGAGTGGTGTC	(SEQ ID NO 24)
MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTCGAGAGTTGTC	(SEQ ID NO 28)
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)
MCH-ICG-3 :	GGTGTGGTCCTTGACTTATGGATAG	(SEQ ID NO 210)
MGO-ICG-5 :	CGTGAGGGGTCATCGTCTGTAG	(SEQ ID NO 33)
MUL-ICG-1 :	GGTTTCGGGATGTTGTCCCACC	(SEQ ID NO 175)
MGV-ICG-1 :	CGACTGAGGTCGACGTGGTGT	(SEQ ID NO 176)
MGV-ICG-2 :	GGTGTTTGAGCATTGAATAGTGGTTGC	(SEQ ID NO 177)
MGV-ICG-3 :	TCGGGCCGCGTGTTCGTCAAA	(SEQ ID NO 211)
MXE-ICG-1 :	GTTGGGCAGCAGGCAGTAACC	(SEQ ID NO 178)
MSI-ICG-1 :	CCGGCAACGGTTACGTGTTC	(SEQ ID NO 179)

MFO-ICG-1 :   TCGTTGGATGGCCTCGCACCT   (SEQ ID NO 180)

MFO-ICG-2 :   ACTTGGCGTGGGATGCGGGAA   (SEQ ID NO 181)

MML-ICG-1 :   CGGATCGATTGAGTGCTTGTCCC   (SEQ ID NO 188)

MML-ICG-2 :   TCTAAATGAACGCACTGCCGATGG   (SEQ ID NO 189)

MCE-ICG-1 :   TGAGGGAGCCCGTGCCTGTA   (SEQ ID NO 190)

MHP-ICG-1 :   CATGTTGGGCTTGATCGGGTGC   (SEQ ID NO 191)

PA-ICG 1 :   TGGTGTGCTGCGTGATCCGAT   (SEQ ID NO 34)

PA-ICG 4 :   TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC

                                                     (SEQ ID NO 37)

PA-ICG 5 :   CTCTTTCACTGGTGATCATTCAAGTCAAG   (SEQ ID NO 38)

MPN-ICG 1 :   ATCGGTGGTAAATTAAACCCAAATCCCTGT   (SEQ ID NO 49)

MPN-ICG 2 :   CAGTTCTGAAAGAACATTTCCGCTTCTTTC   (SEQ ID NO 50)

MGE-ICG 1 :   CACCCATTAATTTTTTCGGTGTTAAAACCC   (SEQ ID NO 51)

Mycoplasma-ICG :   CAAAACTGAAAACGACAATCTTTCTAGTTCC   (SEQ ID NO 52)

STAU-ICG 1 :   TACCAAGCAAAACCGAGTGAATAAAGAGTT   (SEQ ID NO 53)

STAU-ICG 2 :   CAGAAGATGCGGAATAACGTGAC   (SEQ ID NO 54)

STAU-ICG 3 :   AACGAAGCCGTATGTGAGCATTTGAC   (SEQ ID NO 55)

STAU-ICG 4 :   GAACGTAACTTCATGTTAACGTTTGACTTAT   (SEQ ID NO 56)

ACI-ICG 1 :   GCTTAAGTGCACAGTGCTCTAAACTGA   (SEQ ID NO 57)

ACI-ICG 2 :   CACGGTAATTAGTGTGATCTGACGAAG   (SEQ ID NO 58)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 76 to 106, 157 to 174, 124, 125, 111 to 115, 139 to 144, or 126 to 130, and with said probes or equivalents being possibly used in combination with any probe detecting at least one of the following organisms: Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis or Bordetella pertussis.

3. Method according to claim 1, wherein said sample is a sample taken from the cerebrospinal fluid, and wherein the set of probes as described in step (iii) comprises at least

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one probe chosen from the following spacer probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)
LIS-ICG 1 :	CAAGTAACCGAGAATCATCTGAAAGTGAATC	(SEQ ID NO 39)
LMO-ICG 1 :	AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG	
		(SEQ ID NO 40)
LMO-ICG 2 :	TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC	(SEQ ID NO 41)
LMO-ICG 3 :	AGGCACTATGCTTGAAGCATCGC	(SEQ ID NO 42)
LISP-ICG 1:	CGTTTTCATAAGCGATCGCACGTT	(SEQ ID NO 212)

and preferably from the following spacer probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)

LIS-ICG 1 :	CAAGTAACCGAGAATCATCTGAAAGTGAATC	(SEQ ID NO 39)
LMO-ICG 3 :	AGGCACTATGCTTGAAGCATCGC	(SEQ ID NO 42)
LISP-ICG 1:	CGTTTTCATAAGCGATCGCACGTT	(SEQ ID NO 212)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 116, 118-121, or 213-215,

and with said probes or equivalents being possibly used in combination with any probe detecting at least one of the following organisms: Neisseria meningitidis, Haemophilus influenzae or Streptococcus pneumoniae.

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4. Method according to claim 1, wherein said sample is originating from the uro-genital tract, and wherein the set of probes as described in step (iii) comprises at least one probe chosen from the following spacer probes:

CHTR-ICG 1 :   GGAAGAAGCCTGAGAAGGTTTCTGAC   (SEQ ID NO 45)

CHTR-ICG 2 :   GCATTTATATGTAAGAGCAAGCATTCTATTTCA   (SEQ ID NO 46)

CHTR-ICG 3 :   GAGTAGCGTGGTGAGGACGAGA   (SEQ ID NO 47)

CHTR-ICG 4 :   GAGTAGCGCGGTGAGGACGAGA   (SEQ ID NO 201)

CHPS-ICG 1 :   GGATAACTGTCTTAGGACGGTTTGAC   (SEQ ID NO 48)

MGE-ICG 1 :   CACCCATTAATTTTTTCGGTGTTAAAACCC   (SEQ ID NO 51)

Mycoplasma-ICG :   CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 122, 123, 197, 124 or 125,

with said probes or equivalents being possibly used in combination with any probe detecting at least one of the following organisms: Neisseria gonorrhoeae, Haemophilus ducreyi or Streptococcus agalactiae.

5. Method according to claim 1, wherein said sample is originating from food, and wherein the set of probes as defined in step (iii) comprises at least one probe chosen from the following spacer probes:

LIS-ICG 1 :   CAAGTAACCGAGAATCATCTGAAAGTGAATC   (SEQ ID NO 39)

LMO-ICG 1 :   AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG

                                                                              (SEQ ID NO 40)

LMO-ICG 2 :   TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC   (SEQ ID NO 41)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

LIV-ICG 1 :   GTTAGCATAAATAGGTAACTATTTATGACACAAGTAAC

                                                                              (SEQ ID NO 43)

LSE-ICG 1 :AGTTAGCATAAGTAGTGTAACTATTTATGACACAAG (SEQ ID NO 44)

LISP-ICG 1:   CGTTTTCATAAGCGATCGCACGTT   (SEQ ID NO 212)

STAU-ICG 1 :   TACCAAGCAAAACCGAGTGAATAAAGAGTT   (SEQ ID NO 53)

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STAU-ICG 2 :	CAGAAGATGCGGAATAACGTGAC	(SEQ ID NO 54)
STAU-ICG 3 :	AACGAAGCCGTATGTGAGCATTTGAC	(SEQ ID NO 55)
STAU-ICG 4 :	GAACGTAACTTCATGTTAACGTTTGACTTAT	(SEQ ID NO 56)
BRU-ICG 1 :	CGTGCCGCCTTCGTTTCTCTTT	(SEQ ID NO 59)
BRU-ICG 2 :	TTCGCTTCGGGGTGGATCTGTG	(SEQ ID NO 60)
BRU-ICG 3 :	GCGTAGTAGCGTTTGCGTCGG	(SEQ ID NO 193)
BRU-ICG 4 :	CGCAAGAAGCTTGCTCAAGCC	(SEQ ID NO 194)
SALM-ICG 1 :	CAAAACTGACTTACGAGTCACGTTTGAG	(SEQ ID NO 61)
SALM-ICG 2 :	GATGTATGCTTCGTTATTCCACGCC	(SEQ ID NO 62)
STY-ICG 1 :	GGTCAAACCTCCAGGGACGCC	(SEQ ID NO 63)
SED-ICG 1 :	GCGGTAATGTGTGAAAGCGTTGCC	(SEQ ID NO 64)
YEC-ICG 1 :	GGAAAAGGTACTGCACGTGACTG	(SEQ ID NO 198)
YEC-ICG 2 :	GACAGCTGAAACTTATCCCTCCG	(SEQ ID NO 199)
YEC-ICG 3 :	GCTACCTGTTGATGTAATGAGTCAC	(SEQ ID NO 200)

and preferably from the following spacer probes:

LIS-ICG 1 :	CAAGTAACCGAGAATCATCTGAAAGTGAATC	(SEQ ID NO 39)
LMO-ICG 3 :	AGGCACTATGCTTGAAGCATCGC	(SEQ ID NO 42)
LISP-ICG 1:	CGTTTTCATAAGCGATCGCACGTT	(SEQ ID NO 212)
STAU-ICG 1 :	TACCAAGCAAAACCGAGTGAATAAAGAGTT	(SEQ ID NO 53)
STAU-ICG 2 :	CAGAAGATGCGGAATAACGTGAC	(SEQ ID NO 54)
STAU-ICG 3 :	AACGAAGCCGTATGTGAGCATTTGAC	(SEQ ID NO 55)
STAU-ICG 4 :	GAACGTAACTTCATGTTAACGTTTGACTTAT	(SEQ ID NO 56)
BRU-ICG 2 :	TTCGCTTCGGGGTGGATCTGTG	(SEQ ID NO 60)
BRU-ICG 3 :	GCGTAGTAGCGTTTGCGTCGG	(SEQ ID NO 193)
BRU-ICG 4 :	CGCAAGAAGCTTGCTCAAGCC	(SEQ ID NO 194)
SALM-ICG 1 :	CAAAACTGACTTACGAGTCACGTTTGAG	(SEQ ID NO 61)
YEC-ICG 1 :	GGAAAAGGTACTGCACGTGACTG	(SEQ ID NO 198)
YEC-ICG 2 :	GACAGCTGAAACTTATCCCTCCG	(SEQ ID NO 199)
YEC-ICG 3 :	GCTACCTGTTGATGTAATGAGTCAC	(SEQ ID NO 200)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the

spacer region sequence corresponding to one of the micro-organisms to be detected in said

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sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 116, 118-121, 213-215, 139-144, 131, 132, 154, 133-138, 195 or 196, with said probes or equivalents being possibly used in combination with any probe detecting strains of <u>Campylobacter</u> species.

6. Method according to claim 1, wherein said sample is originating from the gastro-intestinal tract of a patient, and wherein the set of probes as defined in step (iii) comprises at least one probe chosen from the following spacer probes:

SALM-ICG 1 : CAAAACTGACTTACGAGTCACGTTTGAG   (SEQ ID NO 61)

SALM-ICG 2 : GATGTATGCTTCGTTATTCCACGCC   (SEQ ID NO 62)

STY-ICG 1 : GGTCAAACCTCCAGGGACGCC   (SEQ ID NO 63)

SED-ICG 1 : GCGGTAATGTGTGAAAGCGTTGCC   (SEQ ID NO 64)

YEC-ICG 1 : GGAAAAGGTACTGCACGTGACTG   (SEQ ID NO 198)

YEC-ICG 2 : GACAGCTGAAACTTATCCCTCCG   (SEQ ID NO 199)

YEC-ICG 3 :   GCTACCTGTTGATGTAATGAGTCAC   (SEQ ID NO 200)

and preferably from the following spacer probes:

SALM-ICG 1 : CAAAACTGACTTACGAGTCACGTTTGAG   (SEQ ID NO 61)

YEC-ICG 1 : GGAAAAGGTACTGCACGTGACTG   (SEQ ID NO 198)

YEC-ICG 2 : GACAGCTGAAACTTATCCCTCCG   (SEQ ID NO 199)

YEC-ICG 3 :   GCTACCTGTTGATGTAATGAGTCAC   (SEQ ID NO 200)

or equivalents of said probes,

and/or wherein the set of probes comprises at least one taxon-specific probe derived from the spacer region sequence corresponding to one of the micro-organisms to be detected in said sample, said spacer region sequence being chosen from any of the sequences as represented by SEQ ID NO 133-138 or 195-196,

with said probes or equivalents being possibly used in combination with any probe detecting <u>Campylobacter</u> species.

7. Method according to claim 1 to detect and identify one or more strains of <u>Mycobacterium</u> species and subspecies in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MYC-ICG-1 :   ACTGGATAGTGGTTGCGAGCATCTA   (SEQ ID NO 1)

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MYC-ICG-22 :   CTTCTGAATAGTGGTTGCGAGCATCT   (SEQ ID NO 2)

MTB-ICG-1 :   GGGTGCATGACAACAAAGTTGGCCA   (SEQ ID NO 3)

MTB-ICG-2 :   GACTTGTTCCAGGTGTTGTCCCAC   (SEQ ID NO 4)

MTB-ICG-3 :   CGGCTAGCGGTGGCGTGTTCT   (SEQ ID NO 5)

MAI-ICG-1 :   CAACAGCAAATGATTGCCAGACACAC   (SEQ ID NO 6)

MIL-ICG-11 :   GAGGGGTTCCCGTCTGTAGTG   (SEQ ID NO 7)

MIL-ICG-22 :   TGAGGGGTTCTCGTCTGTAGTG   (SEQ ID NO 8)

MAC-ICG-1 :   CACTCGGTCGATCCGTGTGGA   (SEQ ID NO 9)

MAV-ICG-1 :   TCGGTCCGTCCGTGTGGAGTC   (SEQ ID NO 10)

MAV-ICG-22 :   GTGGCCGGCGTTCATCGAAA   (SEQ ID NO 11)

MIN-ICG-1 :   GCATAGTCCTTAGGGCTGATGCGTT   (SEQ ID NO 12)

MIN-ICG-2 :   GCTGATGCGTTCGTCGAAATGTGTA   (SEQ ID NO 13)

MIN-ICG-22 :   CTGATGCGTTCGTCGAAATGTGT   (SEQ ID NO.14)

MIN-ICG-222 : TGATGCGTTCGTCGAAATGTGT   (SEQ ID NO 15)

MIN-ICG-2222 : GGCTGATGCGTTCGTCGAAATGTGTAA   (SEQ ID NO 16)

MAL-ICG-1 :   ACTAGATGAACGCGTAGTCCTTGT   (SEQ ID NO 17)

MHEF-ICG-1 :   TGGACGAAAACCGGGTGCACAA   (SEQ ID NO 18)

MAH-ICG-1 :   GTGTAATTTCTTTTTTAACTCTTGTGTGTAAGTAAGTG

                                                         (SEQ ID NO 19)

MCO-ICG-11 :   TGGCCGGCGTGTTCATCGAAA   (SEQ ID NO 20)

MTH-ICG-11 :   GCACTTCAATTGGTGAAGTGCGAGCC   (SEQ ID NO 21)

MTH-ICG-2 :   GCGTGGTCTTCATGGCCGG   (SEQ ID NO 22)

MEF-ICG-11 :   ACGCGTGGTCCTTCGTGG   (SEQ ID NO 23)

MSC-ICG-1 :   TCGGCTCGTTCTGAGTGGTGTC   (SEQ ID NO 24)

MKA-ICG-1 :   GATGCGTTTGCTACGGGTAGCGT   (SEQ ID NO 25)

MKA-ICG-2 :   GATGCGTTGCCTACGGGTAGCGT   (SEQ ID NO 26)

MKA-ICG-3 :   ATGCGTTGCCCTACGGGTAGCGT   (SEQ ID NO 27)

MKA-ICG-4 :   CGGGCTCTGTTCGAGAGTTGTC   (SEQ ID NO 28)

MKA-ICG-5 :   CCCTCAGGGATTTTCTGGGTGTTG   (SEQ ID NO 182)

MKA-ICG-6 :   GGACTCGTCCAAGAGTGTTGTCC   (SEQ ID NO 183)

MKA-ICG-7 :   TCGGGCTTGGCCAGAGCTGTT   (SEQ ID NO 184)

MKA-ICG-8 :   GGGTGCGCAACAGCAAGCGA   (SEQ ID NO 185)

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MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)
MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)
MGO-ICG-1 :	AACACCCTCGGGTGCTGTCC	(SEQ ID NO 31)
MGO-ICG-2 :	GTATGCGTTGTCGTTCGCGGC	(SEQ ID NO 32)
MGO-ICG-5 :	CGTGAGGGGTCATCGTCTGTAG	(SEQ ID NO 33)
MUL-ICG-1 :	GGTTTCGGGATGTTGTCCCACC	(SEQ ID NO 175)
MGV-ICG-1 :	CGACTGAGGTCGACGTGGTGT	(SEQ ID NO 176)
MGV-ICG-2 :	GGTGTTTGAGCATTGAATAGTGGTTGC	(SEQ ID NO 177)
MXE-ICG-1 :	GTTGGGCAGCAGGCAGTAACC	(SEQ ID NO 178)
MSI-ICG-1 :	CCGGCAACGGTTACGTGTTC	(SEQ ID NO 179)
MFO-ICG-1 :	TCGTTGGATGGCCTCGCACCT	(SEQ ID NO 180)
MFO-ICG-2 :	ACTTGGCGTGGGATGCGGGAA	(SEQ ID NO 181)
MML-ICG-1 :	CGGATCGATTGAGTGCTTGTCCC	(SEQ ID NO 188)
MML-ICG-2 :	TCTAAATGAACGCACTGCCGATGG	(SEQ ID NO 189)
MCE-ICG-1 :	TGAGGGAGCCCGTGCCTGTA	(SEQ ID NO 190)
MHP-ICG-1 :	CATGTTGGGCTTGATCGGGTGC	(SEQ ID NO 191)

and more preferably to at least one probe of the following restricted group of spacer probes:

MYC-ICG-1 :	ACTGGATAGTGGTTGCGAGCATCTA	(SEQ ID NO 1)
MYC-ICG-22 :	CTTCTGAATAGTGGTTGCGAGCATCT	(SEQ ID NO 2)
MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)
MAI-ICG-1 :	CAACAGCAAATGATTGCCAGACACAC	(SEQ ID NO 6)
MIL-ICG-11 :	GAGGGGTTCCCGTCTGTAGTG	(SEQ ID NO 7)
MIL-ICG-22 :	TGAGGGGTTCTCGTCTGTAGTG	(SEQ ID NO 8)
MAC-ICG-1 :	CACTCGGTCGATCCGTGTGGA	(SEQ ID NO 9)
MAV-ICG-1 :	TCGGTCCGTCCGTGTGGAGTC	(SEQ ID NO 10)
MAV-ICG-22 :	GTGGCCGGCGTTCATCGAAA	(SEQ ID NO 11)
MIN-ICG-1 :	GCATAGTCCTTAGGGCTGATGCGTT	(SEQ ID NO 12)
MAL-ICG-1 :	ACTAGATGAACGCGTAGTCCTTGT	(SEQ ID NO 17)

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MCO-ICG-11 :   TGGCCGGCGTGTTCATCGAAA   (SEQ ID NO 20)

MTH-ICG-11 :   GCACTTCAATTGGTGAAGTGCGAGCC   (SEQ ID NO 21)

MTH-ICG-2 :   GCGTGGTCTTCATGGCCGG   (SEQ ID NO 22)

MEF-ICG-11 :   ACGCGTGGTCCTTCGTGG   (SEQ ID NO 23)

MSC-ICG-1 :   TCGGCTCGTTCTGAGTGGTGTC   (SEQ ID NO 24)

MKA-ICG-3 :   ATGCGTTGCCCTACGGGTAGCGT   (SEQ ID NO 27)

MKA-ICG-4 :   CGGGCTCTGTTCGAGAGTTGTC   (SEQ ID NO 28)

MKA-ICG-5 :   CCCTCAGGGATTTTCTGGGTGTTG   (SEQ ID NO 182)

MKA-ICG-6 :   GGACTCGTCCAAGAGTGTTGTCC   (SEQ ID NO 183)

MKA-ICG-7 :   TCGGGCTTGGCCAGAGCTGTT   (SEQ ID NO 184)

MKA-ICG-8 :   GGGTGCGCAACAGCAAGCGA   (SEQ ID NO 185)

MKA-ICG-9 :   GATGCGTTGCCCCTACGGG   (SEQ ID NO 186)

MKA-ICG-10 :   CCCTACGGGTAGCGTGTTCTTTTG   (SEQ ID NO 187)

MCH-ICG-1 :   GGTGTGGACTTTGACTTCTGAATAG   (SEQ ID NO 29)

MCH-ICG-2 :   CGGCAAAACGTCGGACTGTCA   (SEQ ID NO 30)

MCH-ICG-3 :   GGTGTGGTCCTTGACTTATGGATAG   (SEQ ID NO 210)

MGO-ICG-5 :   CGTGAGGGGTCATCGTCTGTAG   (SEQ ID NO 33)

MUL-ICG-1 :   GGTTTCGGGATGTTGTCCCACC   (SEQ ID NO 175)

MGV-ICG-1 :   CGACTGAGGTCGACGTGGTGT   (SEQ ID NO 176)

MGV-ICG-2 :   GGTGTTTGAGCATTGAATAGTGGTTGC   (SEQ ID NO 177)

MGV-ICG-3 :   TCGGGCCGCGTGTTCGTCAAA   (SEQ ID NO 211)

MXE-ICG-1 :   GTTGGGCAGCAGGCAGTAACC   (SEQ ID NO 178)

MSI-ICG-1 :   CCGGCAACGGTTACGTGTTC   (SEQ ID NO 179)

MFO-ICG-1 :   TCGTTGGATGGCCTCGCACCT   (SEQ ID NO 180)

MFO-ICG-2 :   ACTTGGCGTGGGATGCGGGAA   (SEQ ID NO 181)

MML-ICG-1 :   CGGATCGATTGAGTGCTTGTCCC   (SEQ ID NO 188)

MML-ICG-2 :   TCTAAATGAACGCACTGCCGATGG   (SEQ ID NO 189)

MCE-ICG-1 :   TGAGGGAGCCCGTGCCTGTA   (SEQ ID NO 190)

MHP-ICG-1 :   CATGTTGGGCTTGATCGGGTGC   (SEQ ID NO 191)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 76-110, or 157-174 provided said probe
hybridizes specifically to a Mycobacterium species.

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8. Method according to claim 7, to detect and identify one or more Mycobacterium tuberculosis complex strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MTB-ICG-1 :	GGGTGCATGACAACAAAGTTGGCCA	(SEQ ID NO 3)
MTB-ICG-2 :	GACTTGTTCCAGGTGTTGTCCCAC	(SEQ ID NO 4)
MTB-ICG-3 :	CGGCTAGCGGTGGCGTGTTCT	(SEQ ID NO 5)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 76 provided said probe hybridizes specifically to the M. tuberculosis complex.

9. Method according to claim 7 to detect and identify one or more Mycobacterium strains from the MAIS-complex, wherein step (iii) comprises hybridizing to at least one of the following probes:

MAI-ICG-1 :	CAACAGCAAATGATTGCCAGACACAC	(SEQ ID NO 6)
MIL-ICG-11 :	GAGGGGTTCCCGTCTGTAGTG	(SEQ ID NO 7)
MIL-ICG-22 :	TGAGGGGTTCTCGTCTGTAGTG	(SEQ ID NO 8)
MAC-ICG-1 :	CACTCGGTCGATCCGTGTGGA	(SEQ ID NO 9)
MAV-ICG-1 :	TCGGTCCGTCCGTGTGGAGTC	(SEQ ID NO 10)
MAV-ICG-22 :	GTGGCCGGCGTTCATCGAAA	(SEQ ID NO 11)
MIN-ICG-1 :	GCATAGTCCTTAGGGCTGATGCGTT	(SEQ ID NO 12)
MIN-ICG-2 :	GCTGATGCGTTCGTCGAAATGTGTA	(SEQ ID NO 13)
MIN-ICG-22 :	CTGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 14)
MIN-ICG-222 :	TGATGCGTTCGTCGAAATGTGT	(SEQ ID NO 15)
MIN-ICG-2222 :	GGCTGATGCGTTCGTCGAAATGTGTAA	(SEQ ID NO 16)
MAL-ICG-1 :	ACTAGATGAACGCGTAGTCCTTGT	(SEQ ID NO 17)
MHEF-ICG-1 :	TGGACGAAAACCGGGTGCACAA	(SEQ ID NO 18)
MAH-ICG-1 :	GTGTAATTTCTTTTTTAACTCTTGTGTGTAAGTAAGTG	
		(SEQ ID NO 19)
MCO-ICG-11 :	TGGCCGGCGTGTTCATCGAAA	(SEQ ID NO 20)
MTH-ICG-11 :	GCACTTCAATTGGTGAAGTGCGAGCC	(SEQ ID NO 21)
MTH-ICG-2 :	GCGTGGTCTTCATGGCCGG	(SEQ ID NO 22)

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MEF-ICG-11 :   ACGCGTGGTCCTTCGTGG   (SEQ ID NO 23)

MSC-ICG-1 :   TCGGCTCGTTCTGAGTGGTGTC   (SEQ ID NO 24)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 77-100 or 108-110, provided said probe

hybridizes specifically to strains from the MAIS complex.

10. Method according to claim 9 to detect and identify one or more <u>M. avium</u> and

<u>M. paratuberculosis</u> strains in a sample, wherein step (iii) comprises hybridizing to at least

one of the following probes:

MAV-ICG-1 :   TCGGTCCGTCCGTGTGGAGTC   (SEQ ID NO 10)

MAV-ICG-22 :   GTGGCCGGCGTTCATCGAAA   (SEQ ID NO 11)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 77 and 78 provided said probe hybridizes

specifically to <u>M</u>. <u>avium</u> or <u>M</u>. <u>paratuberculosis</u>.

11. Method according to claim 9 to detect and identify one or more <u>Mycobacterium</u>

<u>intracellulare</u> strains and MIC-strains in a sample, wherein step (iii) comprises hybridizing

to at least one of the following probes:

MAI-ICG-1 :   CAACAGCAAATGATTGCCAGACACAC   (SEQ ID NO 6)

MIL-ICG-11 :   GAGGGGTTCCCGTCTGTAGTG   (SEQ ID NO 7)

MIL-ICG-22 :   TGAGGGGTTCTCGTCTGTAGTG   (SEQ ID NO 8)

MAC-ICG-1 :   CACTCGGTCGATCCGTGTGGA   (SEQ ID NO 9)

MIN-ICG-1 :   GCATAGTCCTTAGGGCTGATGCGTT   (SEQ ID NO 12)

MIN-ICG-2 :   GCTGATGCGTTCGTCGAAATGTGTA   (SEQ ID NO 13)

MIN-ICG-22 :   CTGATGCGTTCGTCGAAATGTGT   (SEQ ID NO 14)

MIN-ICG-222 :   TGATGCGTTCGTCGAAATGTGT   (SEQ ID NO 15)

MIN-ICG-2222 :   GGCTGATGCGTTCGTCGAAATGTGTAA   (SEQ ID NO 16)

MAL-ICG-1 :   ACTAGATGAACGCGTAGTCCTTGT   (SEQ ID NO 17)

MHEF-ICG-1 :   TGGACGAAAACCGGGTGCACAA   (SEQ ID NO 18)

MAH-ICG-1 :   GTGTAATTTCTTTTTTAACTCTTGTGTGTAAGTAAGTG

                                                            (SEQ ID NO 19)

MCO-ICG-11 :   TGGCCGGCGTGTTCATCGAAA   (SEQ ID NO 20)

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MTH-ICG-11 : GCACTTCAATTGGTGAAGTGCGAGCC   (SEQ ID NO 21)

MTH-ICG-2 :   GCGTGGTCTTCATGGCCGG   (SEQ ID NO 22)

MEF-ICG-11 : ACGCGTGGTCCTTCGTGG   (SEQ ID NO 23),

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 provided said probe hybridizes specifically to M. intracellulare strains and MIC-strains.

12. Method according to claim 9 to detect and identify one or more Mycobacterium intracellulare strains in a sample, wherein step (iii) comprises hybrdizing to the following probe:

MIN-ICG-1 : GCATAGTCCTTAGGGCTGATGCGTT   (SEQ ID NO 12),

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 89 provided said probe hybridizes specifically to M. intracellulare.

13. Method according to claim 9 to detect and identify one or more Mycobacterium scrofulaceum strains in a sample, wherein step (iii) comprises hybrdizing to the following probe:

MSC-ICG-1 :   TCGGCTCGTTCTGAGTGGTGTC   (SEQ ID NO 24),

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 100 provided said probe hybridizes specifically to M. scrofulaceum.

14. Method according to claim 7 to detect and identify one or more Mycobacterium kansasii strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MKA-ICG-1 :   GATGCGTTTGCTACGGGTAGCGT   (SEQ ID NO 25)

MKA-ICG-2 :   GATGCGTTGCCTACGGGTAGCGT   (SEQ ID NO 26)

MKA-ICG-3 :   ATGCGTTGCCCTACGGGTAGCGT   (SEQ ID NO 27)

MKA-ICG-4 :   CGGGCTCTGTTCGAGAGTTGTC   (SEQ ID NO 28)

MKA-ICG-5 :   CCCTCAGGGATTTTCTGGGTGTTG   (SEQ ID NO 182)

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MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)

and more preferably to:

MKA-ICG-3 :	ATGCGTTGCCCTACGGGTAGCGT	(SEQ ID NO 27)
MKA-ICG-4 :	CGGGCTCTGTTCGAGAGTTGTC	(SEQ ID NO 28),
MKA-ICG-5 :	CCCTCAGGGATTTTCTGGGTGTTG	(SEQ ID NO 182)
MKA-ICG-6 :	GGACTCGTCCAAGAGTGTTGTCC	(SEQ ID NO 183)
MKA-ICG-7 :	TCGGGCTTGGCCAGAGCTGTT	(SEQ ID NO 184)
MKA-ICG-8 :	GGGTGCGCAACAGCAAGCGA	(SEQ ID NO 185)
MKA-ICG-9 :	GATGCGTTGCCCCTACGGG	(SEQ ID NO 186)
MKA-ICG-10 :	CCCTACGGGTAGCGTGTTCTTTTG	(SEQ ID NO 187)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 101, 167, 168, or 169 provided said probe hybridizes specifically to <u>M</u>. <u>kansasii</u>.

15. Method according to claim 7 to detect and identify one or more <u>Mycobacterium</u> <u>chelonae</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MCH-ICG-1 :	GGTGTGGACTTTGACTTCTGAATAG	(SEQ ID NO 29)
MCH-ICG-2 :	CGGCAAAACGTCGGACTGTCA	(SEQ ID NO 30)
MCH-ICG-3 :	GGTGTGGTCCTTGACTTATGGATAG	(SEQ ID NO 210)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 102, 103 or 174 provided said probe hybridizes specifically to <u>M</u>. <u>chelonae</u>.

16. Method according to claim 7 to detect and identify one or more <u>Mycobacterium</u> <u>gordonae</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

| MGO-ICG-1 : | AACACCCTCGGGTGCTGTCC | (SEQ ID NO 31) |

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MGO-ICG-2 :   GTATGCGTTGTCGTTCGCGGC   (SEQ ID NO 32)

MGO-ICG-5 :   CGTGAGGGGTCATCGTCTGTAG   (SEQ ID NO 33)

and more preferably to:

MGO-ICG-5 :   CGTGAGGGGTCATCGTCTGTAG   (SEQ ID NO 33),

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 104, 105 or 106 provided said probe hybridizes specifically to M. gordonae.

17. Method according to claim 7 to detect and identify one or more Mycobacterium ulcerans strains or M. marinum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MUL-ICG-1 :   GGTTTCGGGATGTTGTCCCACC   (SEQ ID NO 175)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 157 provided said probe hybridizes specifically to M. ulcerans and M. marinum.

18. Method according to claim 7 to detect and identify one or more Mycobacterium genavense strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MGV-ICG-1 :   CGACTGAGGTCGACGTGGTGT   (SEQ ID NO 176)

MGV-ICG-2 :   GGTGTTTGAGCATTGAATAGTGGTTGC   (SEQ ID NO 177)

MGV-ICG-3 :   TCGGGCCGCGTGTTCGTCAAA   (SEQ ID NO 211)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 158, 159, 160, 161 or 162 provided said probe hybridizes specifically to M. genavense.

19. Method according to claim 7 to detect and identify one or more Mycobacterium xenopi strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MXE-ICG-1 :   GTTGGGCAGCAGGCAGTAACC   (SEQ ID NO 178)

or to equivalents of said probe,

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and/or to any probe derived from SEQ ID NO 163, provided said probe hybridizes specifically to M. xenopi.

20. Method according to claim 7 to detect and identify one or more Mycobacterium simiae strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MSI-ICG-1 : CCGGCAACGGTTACGTGTTC   (SEQ ID NO 179)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 164 or 165 provided said probe hybridizes specifically to M. simiae.

21. Method according to claim 7 to detect and identify one or more Mycobacterium fortuitum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MFO-ICG-1 :   TCGTTGGATGGCCTCGCACCT   (SEQ ID NO 180)

MFO-ICG-2 :   ACTTGGCGTGGGATGCGGGAA   (SEQ ID NO 181)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 166, provided said probe hybridizes specifically to M. fortuitum.

22. Method according to claim 7 to detect and identify one or more Mycobacterium celatum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MCE-ICG-1 :   TGAGGGAGCCCGTGCCTGTA   (SEQ ID NO 190)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 170, provided said probe hybridizes specifically to M. celatum.

23. Method according to claim 7 to detect and identify one or more Mycobacterium haemophilum strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

MHP-ICG-1 :   CATGTTGGGCTTGATCGGGTGC   (SEQ ID NO 191)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 171, 172 or 173, provided said probe hybridizes specifically to M. haemophilum,

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24. Method according to claim 7 to detect and identify one or more <u>Mycobacterium</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MYC-ICG-1 :   ACTGGATAGTGGTTGCGAGCATCTA   (SEQ ID NO 1)

MYC-ICG-22 :   CTTCTGAATAGTGGTTGCGAGCATCT   (SEQ ID NO 2)

or to equivalents of said probes.

25. Method according to claim 1 to detect and identify one or more <u>Mycoplasma</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MPN-ICG 1 :   ATCGGTGGTAAATTAAACCCAAATCCCTGT   (SEQ ID NO 49)

MPN-ICG 2 :   CAGTTCTGAAAGAACATTTCCGCTTCTTTC   (SEQ ID NO 50)

MGE-ICG 1 :   CACCCATTAATTTTTTCGGTGTTAAAACCC   (SEQ ID NO 51)

Mycoplasma-ICG :   CAAAACTGAAAACGACAATCTTTCTAGTTCC (SEQ ID NO 52)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID N0 124 or 125 provided said probe hybridizes specifically with <u>Mycoplasma</u> species.

26. Method according to claim 25 to detect and identify one or more <u>Mycoplasma</u> <u>pneumoniae</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

MPN-ICG 1 :   ATCGGTGGTAAATTAAACCCAAATCCCTGT   (SEQ ID NO 49)

MPN-ICG 2 :   CAGTTCTGAAAGAACATTTCCGCTTCTTTC   (SEQ ID NO 50)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 125 provided said probe hybridizes specifically to <u>Mycoplasma pneumoniae.</u>

27. Method according to claim 25 to detect and identify one or more <u>Mycoplasma</u> <u>genitalium</u> strains in a sample, wherein step (iii) comprises hybridizing to the following probe:

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MGE-ICG 1 :   CACCCATTAATTTTTTCGGTGTTAAAACCC   (SEQ ID NO 51)

or to equivalents of said probe,

or to any probe derived from SEQ ID NO 124 provided said probe hybridizes specifically

to <u>Mycoplasma genitalium</u>.

28. Method according to claim 1 to detect and identify one or more <u>Pseudomonas</u>

strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following

probes:

PA-ICG 1 :	TGGTGTGCTGCGTGATCCGAT	(SEQ ID NO 34)
PA-ICG 2 :	TGAATGTTCGTGGATGAACATTGATT	(SEQ ID NO 35)
PA-ICG 3 :	CACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 36)
PA-ICG 4 :	TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	
		(SEQ ID NO 37)
PA-ICG 5 :	CTCTTTCACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 38),

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 111, 112, 113, 114 or 115 provided said

probe hybridizes specifically to <u>Pseudomonas</u> strains.

29. Method according to claim 28 to detect and identify one or more <u>Pseudomonas</u>

<u>aeruginosa</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the

following probes:

PA-ICG 1 :	TGGTGTGCTGCGTGATCCGAT	(SEQ ID NO 34)
PA-ICG 2 :	TGAATGTTCGTGGATGAACATTGATT	(SEQ ID NO 35)
PA-ICG 3 :	CACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 36)
PA-ICG 4 :	TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	
		(SEQ ID NO 37)
PA-ICG 5 :	CTCTTTCACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 38),

and most preferably to at least one of the following probes:

PA-ICG 1 :	TGGTGTGCTGCGTGATCCGAT	(SEQ ID NO 34)
PA-ICG 4 :	TGAATGTTCGT(G/A)(G/A)ATGAACATTGATTTCTGGTC	
		(SEQ ID NO 37)
PA-ICG 5 :	CTCTTTCACTGGTGATCATTCAAGTCAAG	(SEQ ID NO 38)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 111 provided said probe hybridizes specifically to Pseudomonas aeruginosa.

30. Method according to claim 1 to detect and identify one or more Staphylococcus species in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

STAU-ICG 1 : TACCAAGCAAAACCGAGTGAATAAAGAGTT (SEQ ID NO 53)
STAU-ICG 2 : CAGAAGATGCGGAATAACGTGAC (SEQ ID NO 54)
STAU-ICG 3 : AACGAAGCCGTATGTGAGCATTTGAC (SEQ ID NO 55)
STAU-ICG 4 : GAACGTAACTTCATGTTAACGTTTGACTTAT (SEQ ID NO 56)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 139, 140, 141, 142 ,143 or 144 provided said probe hybridizes specifically to Staphylococcus species.

31. Method according to claim 30 to detect and identify one or more Staphylococcus aureus strains, wherein step (iii) comprises hybridizing to at least one, and preferably both of the following probes:

STAU-ICG 3 : AACGAAGCCGTATGTGAGCATTTGAC (SEQ ID NO 55)
STAU-ICG 4 : GAACGTAACTTCATGTTAACGTTTGACTTAT (SEQ ID NO 56),

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 139, 140, 141, 142 or 143 provided said probe hybridizes specifically to Staphylococcus aureus.

32. Method according to claim 30 to detect and identify one or more Staphylococcus epidermidis strains in a sample, wherein step (iii) comprises hybridizing to any probe derived from SEQ ID NO 144 provided said probe hybridizes specifically to Staphylococcus epidermidis.

33. Method according to claim 1 to detect and identify one or more Acinetobacter strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

ACI-ICG 1 :   GCTTAAGTGCACAGTGCTCTAAACTGA   (SEQ ID NO 57)

ACI-ICG 2 :   CACGGTAATTAGTGTGATCTGACGAAG   (SEQ ID NO 58),

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 126, 127, 128, 129 or 130 provided said

probe hybridizes specifically to <u>Acinetobacter</u> sp..

34. Method according to claim 33 to detect and identify one or more <u>Acinetobacter</u>

<u>baumanii</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the

ollowing probes:

ACI-ICG 1 :   GCTTAAGTGCACAGTGCTCTAAACTGA   (SEQ ID NO 57)

ACI-ICG 2 :   CACGGTAATTAGTGTGATCTGACGAAG   (SEQ ID NO 58)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 126 provided said probe hybridizes

specifically to <u>Acinetobacter baumanii</u>.

35. Method according to claim 1 to detect and identify one or more <u>Listeria</u> strains

in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

LIS-ICG 1 :   CAAGTAACCGAGAATCATCTGAAAGTGAATC   (SEQ ID NO 39)

LMO-ICG 1 :   AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG

                                                           (SEQ ID NO 40)

LMO-ICG 2 :   TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC   (SEQ ID NO 41)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

LIV-ICG 1 :   GTTAGCATAAATAGGTAACTATTTATGACACAAGTAAC

                                                           (SEQ ID NO 43)

LSE-ICG 1 :   AGTTAGCATAAGTAGTGTAACTATTTATGACACAAG

LISP-ICG 1:   CGTTTTCATAAGCGATCGCACGTT   (SEQ ID NO 212)

and most preferably to at least one of the following probes:

LIS-ICG 1 :   CAAGTAACCGAGAATCATCTGAAAGTGAATC   (SEQ ID NO 39)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

LISP-ICG 1:   CGTTTTCATAAGCGATCGCACGTT   (SEQ ID NO 212)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 116, 118, 119, 120, 121, 213, 214 or 215 provided said probe hybridizes specifically to <u>Listeria</u> species.

36. Method according to claim 35 to detect and identify one or more <u>Listeria</u> <u>monocytogenes</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

LMO-ICG 1 :   AAACAACCTTTACTTCGTAGAAGTAAATTGGTTAAG

(SEQ ID NO 40)

LMO-ICG 2 :   TGAGAGGTTAGTACTTCTCAGTATGTTTGTTC   (SEQ ID NO 41)

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

and most preferably to the following probe:

LMO-ICG 3 :   AGGCACTATGCTTGAAGCATCGC   (SEQ ID NO 42)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 118 or 120 provided said probe hybridizes specifically to <u>Listeria</u> <u>monocytogenes</u>.

37. Method according to claim 1 to detect and identify one or more <u>Brucella</u> strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

BRU-ICG 1 :   CGTGCCGCCTTCGTTTCTCTTT   (SEQ ID NO 59)

BRU-ICG 2 :   TTCGCTTCGGGGTGGATCTGTG   (SEQ ID NO 60)

BRU-ICG 3 :   GCGTAGTAGCGTTTGCGTCGG   (SEQ ID NO 193)

BRU-ICG 4 :   CGCAAGAAGCTTGCTCAAGCC   (SEQ ID NO 194)

and most preferably to the following probe:

BRU-ICG 2 :   TTCGCTTCGGGGTGGATCTGTG   (SEQ ID NO 60)

BRU-ICG 3 :   GCGTAGTAGCGTTTGCGTCGG   (SEQ ID NO 193)

BRU-ICG 4 :   CGCAAGAAGCTTGCTCAAGCC   (SEQ ID NO 194)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 131, 132 or 154 provided said probe hybridizes specifically to <u>Brucella</u> strains.

38. Method according to claim 1 to detect and identify one or more <u>Salmonella</u> strains

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in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

SALM-ICG 1 :   CAAAACTGACTTACGAGTCACGTTTGAG   (SEQ ID NO 61)

SALM-ICG 2 :   GATGTATGCTTCGTTATTCCACGCC   (SEQ ID NO 62)

STY-ICG 1 :   GGTCAAACCTCCAGGGACGCC   (SEQ ID NO 63)

SED-ICG 1 :   GCGGTAATGTGTGAAAGCGTTGCC   (SEQ ID NO 64)

and most preferably to the following probe:

SALM-ICG 1 :   CAAAACTGACTTACGAGTCACGTTTGAG   (SEQ ID NO 61)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 133, 134, 135, 136, 137 or 138 provided said probe hybridizes specifically to Salmonella strains.

39. Method according to claim 1 to detect and identify one or more Chlamydia strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

CHTR-ICG 1 :   GGAAGAAGCCTGAGAAGGTTTCTGAC   (SEQ ID NO 45)

CHTR-ICG 2 :   GCATTTATATGTAAGAGCAAGCATTCTATTTCA   (SEQ ID NO 46)

CHTR-ICG 3 :   GAGTAGCGTGGTGAGGACGAGA   (SEQ ID NO 47)

CHTR-ICG 4 :   GAGTAGCGCGGTGAGGACGAGA   (SEQ ID NO 201)

CHPS-ICG 1 :   GGATAACTGTCTTAGGACGGTTTGAC   (SEQ ID NO 48)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 122, 123 or 197 provided that said probe hybridizes specifically to Chlamydia strains.

40. Method according to claim 39 to detect and identify one or more Chlamydia trachomatis strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes:

CHTR-ICG 1 :   GGAAGAAGCCTGAGAAGGTTTCTGAC   (SEQ ID NO 45)

CHTR-ICG 2 :   GCATTTATATGTAAGAGCAAGCATTCTATTTCA

                                                     (SEQ ID NO 46)

CHTR-ICG 3 :   GAGTAGCGTGGTGAGGACGAGA   (SEQ ID NO 47)

CHTR-ICG 4 :   GAGTAGCGCGGTGAGGACGAGA   (SEQ ID NO 201)

or to equivalents of said probes,

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and/or to any probe derived from SEQ ID NO 123 or 197 provided said probe hybridizes specifically to Chlamydia trachomatis.

41. Method according to claim 39 to detect and identify one or more Chlamydia psittaci strains in a sample, wherein step (iii) comprises hybridizing to at least the following probe:

CHPS-ICG 1 :   GGATAACTGTCTTAGGACGGTTTGAC   (SEQ ID NO 48)

or to equivalents of said probe,

and/or to any probe derived from SEQ ID NO 122 provided said probe hybridizes specifically to Chlamydia psittaci.

42. Method according to claim 1 to detect one or more Streptococcus strains in a sample, wherein step (iii) comprises hybridizing to any probe derived from SEQ ID NO 145, 146, 147, 148, 149, 150, 151, 152 or 153 provided said probe hybridizes specifically to Streptococcus strains.

43. Method according to claim 1, to detect and identify specifically Chlamydia trachomatis in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers:

    CHTR-P1:   AAGGTTTCTGACTAGGTTGGGC   (SEQ ID NO 69)

    CHTR-P2: GGTGAAGTGCTTGCATGGATCT   (SEQ ID NO 70)

or equivalents of these primers, said equivalents differing in sequence from the above mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region or part of it of Chlamydia trachomatis.

44. Method according to claim 1, to detect and identify specifically Listeria species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers:

    LIS-P1 : ACCTGTGAGTTTTCGTTCTTCTC   (SEQ ID NO 71)

    LIS-P2 : CTATTTGTTCAGTTTTGAGAGGTT   (SEQ ID NO 72)

    LIS-P3 : ATTTTCCGTATCAGCGATGATAC   (SEQ ID NO 73)

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LIS-P4 :	ACGAAGTAAAGGTTGTTTTTCT	(SEQ ID NO 74)
LIS-P5 :	GAGAGGTTACTCTCTTTTATGTCAG	(SEQ ID NO 75)
LIS-P6 :	CTTTTATGTCAGATAAAGTATGCAA	(SEQ ID NO 202)
LIS-P7 :	CGTAAAAGGGTATGATTATTTG	(SEQ ID NO 203)

or equivalents of these primers, said equivalents differing in sequence from the above
mentioned primers by changing one or more nucleotides, provided that said equivalents still
amplify specifically the spacer region or part of it of <u>Listeria</u> species.

45. Method according to claim 1, to detect and identify specifically <u>Mycobacterium</u>
species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer
region or a part of it, using at least one of the following primers:

MYC-P1:	TCCCTTGTGGCCTGTGTG	(SEQ ID NO 65)
MYC-P2:	TCCTTCATCGGCTCTCGA	(SEQ ID NO 66)
MYC-P3:	GATGCCAAGGCATCCACC	(SEQ ID NO 67)
MYC-P4:	CCTCCCACGTCCTTCATCG	(SEQ ID NO 68)
MYC-P5:	CCTGGGTTTGACATGCACAG	(SEQ ID NO 192)

or equivalents of these primers, said equivalents differing in sequence from the above
mentioned primers by changing one or more nucleotides, provided that said equivalents still
amplify specifically the spacer region or part of it of <u>Mycobacterium</u> species.

46. Composition comprising at least one of the probes or primers as defined in claims
1 to 45 and 51 to 53.

47. Probe as defined in any of claims 1 to 42 and 51.

48. Primer as defined in any of claims 43 to 45 and 52 to 53.

49. Reverse hybridization method comprising any of the probes as defined in claims
1 to 42 and 51 wherein said probes are immobilized on a known location on a solid support,
more preferably on a membrane strip.

50. Kit for the detection and identification of at least one micro-organism, or the

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simultaneous detection and identification of several micro-organisms in a sample, comprising the following components:

(i)   when appropiate, at least one suitable primer pair to allow amplification of the 16S-23S rRNA spacer region, or a part of it;

(ii)   at least one of the probes as defined in claims 1 to 42 and 51;

(iii)   a buffer, or components necessary to produce the buffer, enabling a hybridization reaction between said probes and the polynucleic acids present in the sample, or the amplified products thereof;

(iv)   a solution, or components necessary for producing the solution, enabling washing of the hybrids formed under the appropiate wash conditions;

(v)   when appropiate, a means for detecting the hybrids resulting from the preceding hybridization.

51. Method according to claim 1 to detect and identify one or more Yersinia enterocolitica strains in a sample, wherein step (iii) comprises hybridizing to at least one of the following probes :

         YEC-ICG 1   : GGAAAAGGTACTGCACGTGACTG   (SEQ ID NO 198)

         YEC-ICG 2   : GACAGCTGAAACTTATCCCTCCG   (SEQ ID NO 199)

         YEC-ICG 3   : GCTACCTGTTGATGTAATGAGTCAC   (SEQ ID NO 200)

or to equivalents of said probes,

and/or to any probe derived from SEQ ID NO 195 or 196, provided said probe hybridizes specifically to Yersinia enterocolitica strains.

52. Method according to claim 1, to detect and identify specifically Brucella species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers :

         BRU-P1 : TCGAGAATTGGAAAGAGGTC   (SEQ ID NO 204)

         BRU-P2 : AAGAGGTCGGATTTATCCG   (SEQ ID NO 205)

         BRU-P3 : TTCGACTGCAAATGCTCG   (SEQ ID NO 206)

         BRU-P4 : TCTTAAAGCCGCATTATGC   (SEQ ID NO 207)

or equivalents of these primers, said equivalents differing in sequence from the above mentioned primers by changing one or more nucleotides, provided that said equivalents still

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amplify specifically the spacer region or part of it of <u>Brucella</u> species.

53. Method according to claim 1, to detect and identify specifically <u>Yersinia</u> <u>enterocolitica</u> species in a sample, wherein step (ii) comprises amplification of the 16S-23S rRNA spacer region or a part of it, using at least one of the following primers :

YEC-P1 : CCTAATGATATTGATTCGCG   (SEQ ID NO 208)

YEC-P2 : ATGACAGGTTAATCCTTACCCC   (SEQ ID NO 209)

or equivalents of these primers, said equivalents differing in sequence from the above mentioned primers by changing one or more nucleotides, provided that said equivalents still amplify specifically the spacer region or part of it of <u>Yersinia</u> <u>enterocolitica</u> species.

Figure 1

AAGGAGCACC ACGAAAACGC CCCAACTGGT GGGGCGTAGG CCGTGAGGGG TTCTTGTCTG TAGTGGGCGA

GAGCCGGGTG CATGACAACA AAGTTGGCCA CCAACACACT GTTGGGTCCT GAGGCAACAC TCGGACTTGT

TCCAGGTGTT GTCCCACCGC CTTGGGTGTG GGGTGTGGTG TTTGAGAACT GGATAGTGGT TGCGAGCATC

AATGGATACG CTGCCGGCTA GCGGGTGGCGT GTTCTTTGTG CAATATTCTT TGGTTTTTGT TGTGT

(SEQ ID NO 76)

Figure 2

AAGGAGCACC ACGAAAAGCA CCCCAACTGG TGGGGTGCGA GCCCGTGAGGG GTTCCCGTCT GTAGTGGACG

GGGGCCGGNT GCGCAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCCGTC

CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA

GATGAGCGCA TGGTCTTCGT GGCCGGGCGTT CATCGAAATG TGTAATTTCT TCCTTAACTC TTGTGTGT

(SEQ ID NO 77)

Figure 3

AAGGAGCACC ACGAAAAGCA CCCCAACTGG TGGGGTGCGA GCCGGTGAGGG GTTCCCGTCT GTAGTGGACG
GGGGCCGGGT GCGCAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAAACAC TCGGTCCGTC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCCGCA TGGTCTTCGT GGCCGGCGTT CATCGAAATG TGTAATTTCT TTTTTAACTC TTGTGTGT

(SEQ ID NO 78)

Figure 4

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AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCCCGTCT GTAGTGGACG
GGGGCCGGNT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGTCCTTGT GGCTGATGCG CTCGTCGAAA TGTGTAATTT CTTCTTTGGT GTNTGTGTGT
```

(SEQ ID NO 79)

Figure 5

AAGGAGCACC ACGAAAAGCA TCCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCG TAGTCCTTTG TGGCTGATGC GTTCATCAAA ATGTGTAATT TCTTTTTGG TTTNTGTGTG
T

(SEQ ID NO 80)

Figure 6

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCCCGTCT GTAGTGGACG

GGGGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC

CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGGTGT TGAGTATTGG ATAGTGGTTG CGAGCATCTA

GATGAGCGCA TAGCCCTTGC GGCTGATGCG TTCGNCGAAA TGTGTAATTT CTTCTCTGGT TTCTGTGTGT

(SEQ ID NO 81)

Figure 7

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AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GNAGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCG TAGTCCTTCG TGGCTGATGC GTTCATCGAA ATGTGTAATT TCTTCTTTGG TTTTGGGTGT
GT
```

(SEQ ID NO 82)

Figure 8

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCCCGTCT GTAGTGGACG
GGGGCCGGGT GCACAACAGC AAATGATCGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGTCCTTTG GGGCTGATGT GTTTCATCAA AATGTGTAAT TTCTTTTTNG GTTTTNGTGT
GT

(SEQ ID NO 83)

Figure 9

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GGAGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCG TAGTCCTTCG TGGCTGATGC GTTCATTGAA ATGTGTAATT TCTTCTCTGG TTTTTGTGTG
T

(SEQ ID NO 84)

Figure 10

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AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCCCGTCT GTAGTGGACG
GGGGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGTCCTTGT GGCTGATGCG CTCGTCGAAA TGTGTAATTT CTTCTTTGGT TTTTGTGTGT
```

(SEQ ID NO 85)

Figure 11

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCCCGTCT GTAGTGGACG
GGGGCCGGGT GCGCAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGGTGGG GTGTTGGTGT TTGAGTATTG GATAGTGGTT GCGAGCATCT
AGATGAGCGC GTAGTCCTTG TGGCTGATGC GTTCGTCGAA ATGTGTAAATT TCTTCTTTGG GTTTTTGTGT
GT

(SEQ ID NO 86)

Figure 12

AAGGAGCACC ACGAAAAGCA CCCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GNAGCCGGNT GCGCAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGNCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTNGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGGGCGCG TAGTCCTTTG TGACTGATGC GTTCATCAAA ATGTGTAATT TCTTTTTTGN NTTTNGTGTG
T

(SEQ ID NO 87)

Figure 13

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GGAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAGCGCA TAGTCCTTTG TGGCTGACGC GTTCATCGAA ATGTGTAATT TCTTCTTTGG TTTTTGTGTG
T

(SEQ ID NO 88)

SUBSTITUTE SHEET (RULE 26)

Figure 14

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTGANGG GTTCCCGTCT GTAGTGGACG

GGGGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC

CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA

GATGAGCGCA TAGTCCTTAG GGCTGATGCG TTCGTCGNAA TGTGTAATTT CTTCTTTGGT TTTTGTGTGT

(SEQ ID NO 89)

Figure 15

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AAGGAGCACC ACGAAAAGCA TCCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATAATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGTGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TAGTCCTTCG TGGCTGACGT GTTCATCGAA ATGTGTAATT TCTTNTNTTA ACTCTTGTGT
GT
```

(SEQ ID NO 90)

Figure 16

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AAGGAGCACC ACGAAAAGCA CCCCAATTGG TGGGGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GGAGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAAACAC TCGGTCAGTC
CGTGTGGTGT CCCTCCATCT TGGTGGTGGG GTGTGGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TAGTCCTTGT GACTGACGTG TTCATCGAAA TGTGTAATTT CTTTTCTAAC TCTTGTGTGT
```

(SEQ ID NO 91)

Figure 17

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGAAC
CGTGTGGAGT CCCTCCATCT TGGTGGTCGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCGTG TTCATCGAAA TGTGTAAATAT CTTCTCTGGT TTTCGGTGTG
T

(SEQ ID NO 92)

Figure 18

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AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGNT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCGTG TTCATCGAAA TGTGTAATTT CTTTTTNNAC TCTTGTGTGT
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(SEQ ID NO 93)

SUBSTITUTE SHEET (RULE 26)

Figure 19

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGAAC
CGTGTGGAGT CCCTCCATCT TGGTGGGTGGG GTGTGGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCCGTG TTCATCGAAA TGTGTAATTT CTTCTTTGGT TTTNGTGTGT

(SEQ ID NO 94)

SUBSTITUTE SHEET (RULE 26)

Figure 20

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TAGTCCTTCG NGGNCNGCGT GTTCATCGAA ATGTGTAATT TCTNTTNTAA CTCTNGTGTG
T

(SEQ ID NO 95)

Figure 21

AAGGAGCACC ACGAAAAGCA TCCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGGTGGG GTGTGGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TAGTCCTTCG GGGCCGGGCGT GTTCATCGAA ATGTGTAATT TCTTTTTTAA CTCCTGTGTG
T

(SEQ ID NO 96)

Figure 22

AAGGAGCACC ACGAAAAGCA CTTCANTTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGAAC
CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCGTG TTCATCGAAA TGTGTAATTT CTTCTTTAAC TCTTGTGTGT

(SEQ ID NO 97)

Figure 23

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG

AAAACCGGGT GCACAACAGN AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC

CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA

GATGAACGCG TGGTCTTCAT GGCCNGCCGTG TTCATCGAAA TGTGTAATTT CTTTTTTAAC TCTTGTGTGT

(SEQ ID NO 98)

Figure 24

AAGGAGCACC ACGAAAAGCA CTTCAATTGG TGAAGTGCGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAACCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCGATC
CGTGTGGAGT CCCTCCATCT TGGTGGGTGGG GTGTGGTGTT TGAGTATTGG ATAGTGGTTG CGAGCATCTA
GATGAACGCG TGGTCTTCAT GGCCGGCGTG TTCATCGAAA TGTGTAATTT CTTTTTTAAC TCTTGTGTGT

(SEQ ID NO 99)

Figure 25

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AAGGAGCACC ACGAAAAGCA CCCCAACTGG TGGGGTGCGA GCCGTGAGGG GTCCTCGCCT GTAGTGGGCG
GGGGCCGGGT GCACAACAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGGCAACAC TCGGCTCGTT
CTGAGTGGTG TCCCTCCATC TTGGTGGTGG GGTGTGGTGT TTGAGTATTG GATAGTGGTT GCGAGCATCT
AAACGGATGC GTGGCCGGCA ACGGTGGCGT GTTCGTTGAA ATGTGTAATT TCTTTTTTGG TTTTTGTGTG
T
```

(SEQ ID NO 100)

Figure 26

AAGGAGCACC ACGAAAAGCA TCCCAACAAG TGGGGGTGCAA NCCGGTGAGGG GTTCTCGTCT GTAGTGGACG
AAAGCCGGGT GCACGACAAC AAGCAAAGCC AGACACACTA TTGGGTCCTG AGGCAACACT CGGGCTCTGT
TCGAGAGTTG TCCCACCATC TTGGTGGTGG GGTGTGGTGT TTGAGAATTG GATAGTGGTT GCGAGCATCA
AATGGATGCG TTGCCCTACG GGTAGCGTGT TCTTTTGTGC AATTTTATTC TTTGGTTTTT GTGT

(SEQ ID NO 101)

Figure 27

AAGGAGCACC ATTTCCCAGT CGATGAACTA GGGAACATAA AGTAGGCATC TGTAGTGGAT ATCTACTTGG

TGAATATGTT TTGTAAATCC TGTCCACCCC GTGGATGGGT AGTCGGCAAA ACGTCGGACT GTCATAAGAA

TTGAAACGCT GGCACACTGT TGGGTCCTGA GGCAACACGT TGTGTTGTCA CCCTGCTTGG TGGTGGGGTG

TGGACTTTGA CTTCTGAATA GTGGTTGCGA GCATCTAAAC ATAGCCTCGC TCGTTTTCGA GTGGGGCTGG

TTTTGCAATT TTA

(SEQ ID NO 102)

Figure 28

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AAGGAGCACC ATTTCCCAGT CGGATGAACT AGGGAACATA AAGTAGGCAT CTGTAGTGGG TATCTTACTTG
GTGAATATGT TTTGTAAATC CTGTCCACCC CCGTGGATGG GTAGTCGGCA AAACGTCGGA CTGTCATAAG
AATTGAAACG CTGGCACACT GTTGGGTCCT GAGGCAACAC GTTGTGTTGT CACCCTGCTT GGTGGTGGGG
TGTGGACTTT GACTTCTGAA TAGTGGTTGC GAGCATCTAA ACATAGCCTC GCTCGTTTTC GAGTGAGGCT
GGTTTTTGCA ATTTTA
```

(SEQ ID NO 103)

Figure 29

AAGGAGCACC ACGAAGAGCA CTCCAATTGG TGGGGTGCGA GCCGTGAGGG GTCATCGTCT GTAGTGGACG

AAGACCGGGT GCACGACAAC AAGCTAAGCC AGACACACTA TTGGGTCCTG AGGCAACACC CTCGGGTGCT

GTCCCCCCAT CTTGGTGGTG GGGTGTGGTG TTTGAGAATT GGATAGTGGT TGCGAGCATC AAAATGTATG

CGTTGTCGTT CTCGGCAACG TGTTCTTTTT GTGCAATTTA TTCTTTGGTT TTTGTAGTGT TTGT

(SEQ ID NO 104)

Figure 30

AAGGAGCACC ACGAAGAGCA CTCCAATTGG TGGGGTGCGA GCCGNGAGGG GTCATCGTCT GTAGTGGACG
AAGACTGGGT GCACGACAAC AAAGCAAGCC AGACACACTA TTGGGTCCTG AGGCAACACC CTCGGGTGCT
GCCCCTCCAT CTTGGTGGTG GGGTGTGGTG TTTGAGAACT GGATAGTGGT TGCCGAGCATC AAAAATGTAT
GCGTTGTCGT TCGCGACAAC GTGTTCTTTT TGTGCAATTT TAATTCTTTT GGTTTTGGTA GTGTTTGT

(SEQ ID NO 105)

Figure 31

AAGGAGCACC ACGAGAAGCA CTCCAATTGG TGGGGTGCAA GCCGTGAGGG GTCATCGTCT GTAGTGGACG
AAGACCGGGT GCACGACAAC AAGCAAAGCC AGACACACTA TTGGGTCCTG AGGCAACACC CTCGGGTGCT
GTCCCCCCAT CTTGGTGGTG GGGTGTGGTG TTTGAGAACT GGATAGTGGT TGCCGAGCATC AAAATGTATG
CGTTGTCGTT CGCGGGCAACG TGTTCTTTTT GTGCAATTTT TATTCTTTGG TTTTTGTAGT GTTTGT

(SEQ ID NO 106)

Figure 32

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AAGGAGCACC  ACGAAAAGCA  CCCCAATTGG  TGGGGTGCAA  GCCGTGAGGG  GTTCCCGCCT  GTAGTGGGCG
GGGCCGGGTG  CGCAACAGCA  AATGATTGCC  AGACACACTA  TTGGGCCCTG  AGGCAACACT  CGGATCGATT
GAGTGCTTGT  CCCCCCATCT  TGGTGGTGGG  GTGTGGTGTT  TGAGAACTGG  ATAGTGGTTG  CGAGCATCTA
AATGAACGCA  CTGCCGATGG  TGGTGTGTTC  GTTTTGTGTA  ATTTTATTCT  TTGGTTTTTG  TGTTTGT
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(SEQ ID NO 107)

Figure 33

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGTNAGGG GTTCTCGTCT GTAGTGGATG

GCAGCCGGGT GCACANCAGC AAATGATTGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGTCAGTC

CGTGTGGAGT CCCTCCATCT TGGTGGTGGG GTGTGGGNGTT TGAGTATTGG ATAGTGGTTG CGANCATCTA

GATGAACGCG TAGTCCTCNG TGGCTGACGT GTTCATCAAA ATGTGTAATT TCTTTTANGG GTTTNGGTGT

CT

(SEQ ID NO 108)

Figure 34

AAGGAGCACC ACGAAAAGCA CTCCAATTGG TGGGGTGCGA GCCGNGAGGG GTTCTCGCCT GTAGTGGNCG
AGGGCCGGAT GCACAACAAC ACATGATTGC CAGACACACT ATTGGGCCCT GANACAACAC TCGGCCAGTC
CGTGTGGTGT CCCTCCATCT TGGTGGTGGG GTGTGGTGTT TGAGTATNGG ATAGTNGTTG NGANCATCTA
AACGGCTGCG TNGNCNNGAA CGGTGGCGTG TTCGNTAAAA TGTGTAATTT CTTTTNNGGT TTGGGTGTNT

(SEQ ID NO 109)

Figure 35

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AAGGAGCACC  ACGAAAAGCA  CTCCAATTGG  TGGGGTGCGA  GCCGTGAGGG  GTTCTCGCCT  GTAGTGGGCG
ANGGCCGGGT  GCACAACAAC  AAATGATTGC  CAGACACACT  ATTGGGCCCT  GAGACAACAC  TCGGCCAGTC
CGTGTGGTGT  CCCNCCATCT  TGGTGGTGGG  GTGTGGTGTT  TGAGTATTGG  ATAGTGGTTG  CGAGCATCTA
AANGGNTGCG  TTGCCGNNAN  CNGTGGCCGTN TTCGNTAAAA  TGTGTAANTT  CTTTTNGGT   TTGTGTGTGT
```

(SEQ ID NO 110)

Figure 36

ATCGAAGATC CCGGCTTCTT CATAAGCTCC CACACGAATT GCTTGATTCA CTGGTTAGAC GATTGGGTCT

GTAGCTCAGT TGGTTAGAGC GCACCCCTGA TAAGGGTGAG GTCGGCAGTT CGAATCTGCC CAGACCCACC

AATTGTTGGT GTGCTGCGTG ATCCGATACG GGGCCATAGC TCAGCTGGGA GAGCGCCTGC TTTGCACGCA

GGAGGTCAGG AGTTCGATCC TCCTTGGCTC CACCATCTAA AACAATCGTC GAAAGCTCAG AAATGAATGT

TCGTGGATGA ACATTGATTT CTGGTCTTTG CACCAGAACT GTTCTTTAAA AATTCGGGTA TGTGATAGAA

GTAAGACTGA ATGATCTCTT TCACTGGTGA TCATTCAAGT CAAGGTAAAA TTTGCGAGTT CAAGCGCGAA

TTTTCGGCGA ATGTCGTCTT CCAGTATAA CCAGATTGCT TGGGGTTATA T

(SEQ ID NO 111)

Figure 37

ATCGAAGACA TCAGCTTCTT CATAAGTATC CACACGAATT GCTTGATTCA TAGTCGAACG AATGCTGTAA
CGCGACCCGT GTTATAGGTC TGTAGCTCAG TTGGTTAGAG CGCACCCCTG ATAAGGGTGA GGTCGGCAGT
TCAAATCTGC CCAGACCTAC CAATTGCTTG GTCGAGAAGA ATACGGGGCC ATAGCTCAGC TGGGAGAGCG
CCTGCCTTGC ACGCAGGAGG TCAGCGGGTC GATCCCGCTT GGCTCCACCA CTCTCCTCGTG TTGCGGGTGAG
TGTTAAAGAG TTCAGAAATG ATGCCGCTTC AGGTTTGTCC TGTTGAGTGC TCTTTTGACC
GGTACGAAAA TCGTTCTTTA AAAATTTGGA TATGTGATAG AAGTGACTGA TTAATTGCTT TCACTGGCAA
TTGATCTGGT CAAGGTAAAA TTTGTAGTTC TCAAGACGCA AATTTTCGGC GAATGTCGTC TTCACGATTG
AGACAGTAAC CAGATTGCTT GGGGTTATAT

(SEQ ID NO 112)

Figure 38

ATCGAAGACA CCGGCTTCGT CATAAGCTCC CACACGAATT GCTTGATTCA CTTGCGAAAG GCGGATTGGGT

TTAGACCCGA GAGTAAACGAT TGGGTCTGTA GCTCAGTTGG TTAGAGCGCA CCCCTGATAA GGGTGAGGTC

GGCAGTTCGA ATCTGCCCAG ACCCACCAAT CGAAGGGGCC ATAGCTCAGC TGGGAGAGCG CCTGCTTTGC

ACGCAGGAGG TCAGCGGTTC GATCCCGCTT GGCTCCACCA TTAACTCTAG TCGCCGAAAG CTCAGAAATG

AGTGTTTACC AGGATGAGGT TGATTGCCTG GGTTGAACAT TGATTTCTGG ACTTTGCGCC AGAACTGTTC

TTTAAAAATT TGGGTATGTG ATAGAAGTAG ACCGATGTGT TGCTTTCACT GGCAGCATGT CGCGTCAAGG

TAAAATTTGC GTGTTCTCTA TGCAAATTTT CGGCGAATGT CGTCTTCACG TTATAGACAG TAACCAGATT

GCTTGGGGTT ATAT

(SEQ ID NO 113)

Figure 39

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ATCGAAGACT TCAGCTTCTT CATAAGTTCC CACACGAATT GCTTGATTCA CTTGCGAAAA GCGATTGGGT
TGAGACCCGA GAGTGACGAT TGGGTCTGTA GCTCAGTTGG TTAGAGCGCA CCCCTGATAA GGGTGAGGTC
GGCAGTTCGA ATCTGCCCAG ACCCACCAAT TGTCGGGATG GCCAGTGTCA AATGGGGCCA TAGCTCAGCT
GGGAGAGCGC CTGCTTTGCA CGCAGGAGGT CAGGAGTTCG ATCCTCCTTG GCTCCACCAT CAACTCACGA
TCGCTGAAAG CTCAGAAATG AACATTGGTA GTTCAATGTT GATTTCTGGT CTTTGCGCCA GAACTGTTCT
TTAAAAATTT GGGTATGTGA TAGAAGTGAC TAACAGCGTG TTTCACTGCA CGTTGTTAAT CAAGGCAAAA
TTTGCGAGTT CAAGCGGCGA TTTTCGGCGA ATGTCGTCTT CACGTTACGA ATCTATAACC AGATTGCTTG
GGGTTATAT
```

(SEQ ID NO 114)

Figure 40

ATCGACGACA TCAGCTGTCT CATAAGCTCC CACACGAATT GCTTGATTCA TTGAAGAAGA CGATTAGGTT
AGCAACCTTC GATTGGGTCT GTAGCTCAGT TGGTTAGAGC GCACCCCTGA TAAGGGTGAG GTCGGGCAGTT
CGAATCTGCC CAGACCCACC AATTTGCTGG GGCCATAGCT CAGCTGGGAG AGCGCCTGCC TTGCACGCAG
GAGGTCAGCG GTTCGATCCC GCTTGGCTCC ACCACCCCGC TTGCCAGTTT GTCAAAGCTT AGAAATGAAT
ATTCGCGTCG AATATTGATT TCTGAACTTT ATCAGAATCG TTCTTTAAAA ATTTGGGTAT GTGATAGAAA
GATAGACTGG ACAGCACTTT CACTGGTGTG TGTTCAGGCT AAGGTAAAAT TTGTGAGTAA TTACAAGTTT
TCGGGCGAATG TTGTCTTCAC AGTATAACCA GATTGCTTGG GGTTATAT

(SEQ ID NO 115)

Figure 41

TAAGGAAAAG GAAACCTGTG AGTTTTCGTT CTTCTCTGTT TGTTCAGTTT TGAGAGGTTA ATTCTTCTCT
ATACTGTTTG TTCTTTGAAA ACTAGATAAG AAAGTTAGTA AAGTTAGCAT AAATAGGTAA CTATTTATGA
CACAAGTAAC CGAGAATCAT CTGAAAGTGA ATCTTTCATC TGATTGGAAG TATCATCGCT GATACGAAAA
ATCAGAAAAA CAACCTTTAC TTCATCGAAG TAAAATT

(SEQ ID NO 116)

Figure 42

CTAAGGAAAA GGAAACCTGT GAGTTTTCGT TCTTCTCTAT TTGTTCAGTT TTGAGAGGTT AGTACTTCTC
AGTATGTTTG TTCTTTGAAA ACTAGATAAG AAAGTTAGTA AAGTTAGCAT AGATAATTTA TTATTTATGA
CACAAGTAAC CGAGAATCAT CTGAAAGTGA ATCTTTCATC TGATTGGAAG TATCATCGCT GATACGGAAA
ATCAGAAAAA CAACCTTTAC TTCGTAGAAG TAAAATT

(SEQ ID NO 117)

SUBSTITUTE SHEET (RULE 26)

Figure 43

TAAGGAAAAG GAAACCTGTG AGTTTTCGTT CTTCTCTGTT TGTTCAGTTT TGAGAGGTTA TTACTTCTCT
GTATGTTTGT TCTTTGAAAA CTAGATAAGA AAGTTAGTAA AGTTAGCATA AGTAGTGTAA CTATTTATGA
CACAAGTAAC CGAGAATCAT CTGAAAGTGA ATCTTTCATC TAATTCGACG TATCATCGCT GATACAGACA
ATTAGAAAAA CAACCTTTAC TTCGACGAAG TAAAATT

(SEQ ID NO 118)

Figure 44

GGCCTATAGC TCAGCTGGTT AGAGCGCACG CCTGATAAGC GTGAGGTCGA TGGTTCGAGT CCATTTAGGC

CCACTTTTTC TTTCTGACAG AAGAAACACT GTATAACCTA TTTAAGGGGC CTTAGCTCAG CTGGGAGAGC

GCCTGCTTTG CACGCAGGAG GTCAGCGGTT CGATCCCCGCT AGGCTCCACC AAAATTGTTC TTTGAAAACT

AGATAAGAAA GTTAGTAAAG TTAGCATAAA TAGGTAACTA TTTATGACAC AAGTAACCGA GAATCATCTG

AAAGTGAATC TTTCATCTGA TTGGAAGTAT CATCGCTGAT ACGAAAAATC AGAAAAACAA CCTTTACTTC

ATCGAAGTAA ATT

(SEQ ID NO 119)

Figure 45

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TAAGGAAAAG GAAACCTGTG AGTTTTCGTT CTTCTCTATT TGTTCAGTTT TGAGAGGTTA CTCTCTTTTA
TGTCAGATAA AGTATGCAAG GCACTATGCT TGAAGCATCG CGCCACTACA TTTTTGACGG GCCTATAGCT
CAGCTGGTTA GAGCGCACGC CTGATAAGCG TGAGGTCGAT GGTTCGAGTC CATTTAGGCC CACTTTTTCT
TTCTGACATA AGAAATACAA ATAATCATAC CCTTTTACGG GGCCTTAGCT CAGCTGGGAG AGCGCCTGCT
TTGCACGCAG GAGGTCAGCG GTTCGATCCC GCTAGGCTCC ACCAAAATTG TTCTTTGAAA ACTAGATAAG
AAAGTTAGTA AAGTTAGCAT AGATAATTTA TTATTTATGA CACAAGTAAC CGAGAATCAT CTGAAAGTGA
ATCTTTCATC TGATTGGAAG TATCATCGCT GATACGGAAA ATCAGAAAAA CAACCTTTAC TTCGTAGAAG
TAAATT
```

(SEQ ID NO 120)

Figure 46

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TAAGGAAAAG GAAACCTGTN AGTTTNCGTN CTTCTCTGTT TGTNCAGTTT TNAGAGGTTA CTCTCTTTNA
TGTCAGATAA AGTACGCACG GCACGTTGCC TTGGGCAAAG AGCCACTACA TTATTGACGG GCCTATAGCT
CAGCTGGTTA GAGCGCACGC CTGATAAGCG TGAGGTCGAT GGTTCGAGTC CATTTAGGCC CACTTTTTCT
TTCTGACAGA AGAAATCATT TGCACATCCT ATTAATAAGG GNCCTTAGCT CAGCTGGGAG AGCGCCTGCT
TTGCACGCAG GAGGTCAGCG GTTCGATCCC GCTAGGCTCC ACCCAAAATT GTTCTTTGAA AACTAGATAA
GAAAGTTAGT AAAGTTAGCA TAAGTAGTAT AACTATTTAT GACACAAGTA ACCGAGAATC ATCTGAAAGT
GAATCTTTCA TCTAATTCGA CGTATCATCG CTGATACAGA CAATTNGAAA AACAACCTTT ACTTCGACGA
AGTAAATT
```

(SEQ ID NO 121)

Figure 47

TAAGGATAAG GATAACTGTC TTAGGACGGT TTGACTAGGT TGGGCAAGCG TTTTTTTAAT CTTGTATTCT
ATTCCTTTTG CATTGTTAAG CGTTGTTTCC AAAACATTTA GTTTACGATC AAGTATGTTA TGTAAATAAT
ATGGTAACAA GTAAATTCAC ATATAATAAT AGACGTTTAA GAATATATGT CTTTAGGTGA TGTTAACTTG
CATGGATCAA TAATTTACA

(SEQ ID NO 122)

Figure 48

TAAGGATAAG GAAGAAGCCT GAGAAGGTTT CTGACTAGGT TGGGCAAGCA TTTATATGTA AGAGCAAGCA

TTCTATTTCA TTTGTGTTGT TAAGAGTAGC GTGGTGAGGA CGAGACATAT AGTTTGTGAT CAAGTATGTT

ATTGTAAAGA AATAATCATG GTAACAAGTA TATTTCACGC ATAATAAATAG ACGTTTAAGA GTATTTGTCT

TTTAGGTGAA GTGCTTGCAT GGATCTATAG AAATTACA

(SEQ ID NO 123)

Figure 49

CAAATGGAGT TTTTATTTTT TATTTATCTT AAACACCCAT TAATTTTTTC GGTGTTAAAA CCCAAATCAA

TGTTTGGTCT CACAACTAAC ACATTTGGTC AGTTTGTATC CAGTTCTGAA AGAATGTTTT TGAACAGTTC

TTTCAAAACT GAAAACGACA ATCTTTCTAG TTCCAAAAAT AAATACCAAA GGATCAATAC AATAAGTTAC

TAAGGGCTTA TGGT

(SEQ ID NO 124)

Figure 50

CTAATGAAGT TTTTTACTTT TTCTTTTCAT CTTTAATAAA GATAAATACT AAACAAAAACA TCAAAATCCA
TTTATTTATC GGTGGTAAAT TAAACCCAAA TCCCTGTTTG GTCTCACAAC TAACATATTT GGTCAGATTG
TATCCAGTTC TGAAAGAACA TTTCCGCTTC TTTCAAAACT GAAAACGACA ATCTTTCTAG TTCCAAATAA
ATACCAAAGG ATCAATACAA TAAGTTACTA AGGGCTTATG GT

(SEQ ID NO 125)

Figure 51

AACGAAAGAT TGACGATTGG TAAGAATCCA CAACAAGTTG TTCTTCATAG ATGTATCTGA GGGTCTGTAG
CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGGGTCA CAAGTTCAAG TCTTGTCAGA CCCACCATGA
CTTTGACTGG TTGAAGTTAT AGATAAAAGA TACATGATTG ATGATGTAAG CTGGGGACTT AGCTTAGTTG
GTAGAGCGCC TGCTTTGCAC GCAGGAGGTC AGGAGTTCGA CTCCACCAGA ACTTAAGATA
AGTTCGGATT ACAGAAATTA GTAAATAAAG ATTGAGATCT TGGTTTATTA ACTTCTGTGA TTTCATTATC
ACGGTAATTA GTGTGATCTG ACGAAGACAC ATTAACTCAT TAACAGATTG GCAAAATTGA GTCTGAAATA
AATTGTTCAC TCAAGAGTTT AGGTTAAGCA ATTAATCTAG ATGAATTGAG AACTAGCAAA TTAACTGAAT
CAAGCGTTTT GGTATGTGAA TTTAGATTGA AGCTGTACAG TGCTTAAGTG CACAGTGCTC TAAAACTGAAA
TGTTGAAGTT ACTAACTTGT AGGTAACATC GACTGTTTGG GGTTGTAT

(SEQ ID NO 126)

Figure 52

AACGAAAGAT TGACGATTGG TAAGAATCCA CGACAAGTTG TTCTTCATAG ATGTATCTGA GGGTCTGTAG

CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGGTCA CAAGTTCAAG TCTTGTCAGA CCCACCATGA

CTTTGACTGG TTGAAGTTAT AGAAAAGAAG ATACATAAACT GATGATGTAA GCTGGGGGACT TAGCTTAGTT

GGTAGAGCGC CTGCTTTGCA CGCAGGAGGT CAGGAGTTCG ACTCTCCCTAG TCTCCACCA

(SEQ ID NO 127)

Figure 53

AACGAAAGAT TGATGGCCGG TAAGAATCCA CAACAAGTTG TTCTTCGAAG ATGTATCTGA GGGTCTGTAG
CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGGTCA CAAGTTCAAG TCTTGTCAGA CCCACCAAAT
CTGAAAGATA TGTCGTTCAT TATGATTAAA GCTGGGGACT TAGCTTAGTT GGTAGAGCGC CTGCTTTGCA
CGCAGGAGGT CAGGAGTTCG ACTCTCCCTAG TCTCCACCA

(SEQ ID NO 128)

Figure 54

AACGAAAGAT TGACGATTGG TAAGAATCCA CAACAAGTTG TTCTTCATGA CGATGTATCT GAGGGTCTGT

AGCTCAGTTG GTTAGAGCAC ACGCTTGATA AGCGTGGGGT CACAAGTTCA AGTCTTGTCA GACCCACCAA

ATCTGACTAA CAAGCATTAT TAAATGCTGA ATACAGAAAA ACAGAGACAT TGACTTATTG ATAAGCTGGG

GACTTAGCTT AGTTGGTAGA GCGCCTGCTT TGCACGCAGG AGGTCAGGAG TTCGACTCTC CTAGTCTCCA

CCA

(SEQ ID NO 129)

55/103

Figure 55

AACGAAAGAT TGGTGACCGG TAAGAATCCA CAACAAGTTG TTCTTCGAAG ATGTATCTGA GGGTCTGTAG

CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGGTCA CAAGTTCAAG TCTTGTCAGA CCCACCACTA

CTGACGAAGT GATGAATAAT CACAAGCTGC TAGATGAAAA GATATGTCGT TCATTATGAT TAAAGCTGGG

GACTTAGCTT AGTTGGTAGA GCGCCTGCTT TGCACGCCAGG AGGTCAGGAG TTCGACTCTC CTAGTCTCCA

CCA

(SEQ ID NO 130)

Figure 56

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TAAGGAAGAT CGAGAATTGG AAAGAGGTCG GATTTATCCG GATGATCCTT CTCCATCTTA TTAGAACATA
GATCGCAGGC CAGTCAGCCT GACGATCGCT TGCAGGCGTG CCGCCTTCGT TTCTCTTTCT TCATTGTTGA
TTGCTCACGG GCCGTACCGC AGCTGACGCT GCTGGCCCTG CGCAGGCGCG GCCCATCAGG GCCGACGGCC
GGTCGGCCTT GCNAAGCTTC GCTTCGGGGT GGATCTGTGG ATCGCGTAGT AGCCGTTTGCG TCGGTATCTG
GGCTTGTAGC TCAGTTGGTT AGAGCACACG CTTGATAAGC GTGGGGTCGG AGGTTCAAGT CCTCCCAGGC
CCACCAAGTT ACTTGATGAG GGGCCGTAGC TCAGCTGGGA GAGCACCTGC TTTGCAAGCA GGGGGTCGTC
GGTTCGATCC CGTCCGGCTC CACCATCATG TTGGTGTTGA GACGGATATT GGCAATCAAC AAAAGAAAGA
AACAAGTTTG CGGACTNTTA CGAAAGTCTG CCTGTTCTGT ATGAAATCGT GAAGAGAAGA TGTAATCGGA
TCAACTGAAG AGTTGATGTC GCAAGAAGCT TGCTCAAGCC TTGCATAAATG ATTGATGTGT TTAACCGCCA
TCACCGATTG TATCTCGAGA AGCTGGTCTT TCTGCTGATA CTGTTGAAAC GAGCATTTGC AGTCGAATGG
CAACATTCGG CGTCGCATAA TGCGGCTTTA AGAGCTGAGT TTTGATGGAT ATTGGCAATG AGAGTGATCA
AGTGTCTTAA GGGCATTGGT GGATGCCCTTG GCATGCAC
```

(SEQ ID NO 131)

Figure 57

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TAAGGAGGAT CGAGAATTGG AAAGAGGCCG GATTTATCCG GATGATCCTT CTCCATCTTA TTAGAACATA
GATCGGCAGNC CAGTCAGCCT GACGATCGCT TGCCAGGCGTG CCGCCTTCGT TTCTCTTTCT TCATTGTTGA
TTGCTCACGG GCCGTACCGC AGCTGACGCT GCTGGCCCTG CGCAGGCGCG GNCCATCAGG GCCGACGGCC
GGTCGGCCTT GCGAAGCTTC GCTTCGGGGT GGATCTGTGG ATCGCGTAGT AGCCGTTTGCG TCGGTATCTG
GGCTTGTAGC TCAGTTGGTT AGAGCACACG CTTGATAAGC GTGGGGTCGG AGGTTCAAGT CCTCCCAGGC
CCACCAAGTT ACTTGATGAG GGGCCGTAGC TCAGCTGGGA TCAGCTGGGA TTTGCAAGCA GGGGGTCGTC
GGTTCGATCC CGTCCGGCTC CACCATCATG TTGGTGTTGA GAGCACCTGC AAAAGAAAGA
AACAAGTTTG CGGACTNTTA CGAAAGTCTG CCTGTTCTGT GACGGATATT GAAGAGAAGA TGTAATCGGA
TCAACTGAAG AGTTGATGTC GCAAGAAGCT TGCTCAAGCC ATGAAATCGT ATTGATGTGT TTAACCGCCA
TCACCGATTG TATCTCGAGA AGCTGGTCTT TCTGCTGATA TTGCATAAATG CTGTTGAAAC GAGCATTTGC AGTCGAATGG
CAACATTCGG CGTCGCATAA TGCGGCTTTA AGAGCTGAGT TTTGATGGAT ATTGGCAATG AGAGTGATCA
AGTGTCTTAA GGGCATTGGT GCATGCAC
```

(SEQ ID NO 132)

Figure 58

CCTTAAAGAA CTGTTCTTTG CAGTGCTCAC ACAGATTGTC TGATGAAAAG TAAATAGCAA GGCGTCTTGC
GAAGCAGACT GATACGTCCC CTTCGTCTAG AGGCCCAGGA CACCGCCCTT TCACGGCGGT AACAGGGGTT
CGAATCCCCT AGGGGACGCC ACTTGCGCGG TAATGTGTGA AAGCGTTGCC ATCAGTATCT CAAAACTGAC
TTACGAGTCA CGTTTGAGAT ATTTGCTCTT TAAAAATCTG GATCAAGCTG AAAATTGAAA CACAGAACAA
CGAAAGTTGT TCGTGAGTCT CTCAAATTTT CGCAACACGA TGATGAATCG TAAGAAACAT CTTCGGGTTG
TGA

(SEQ ID NO 133)

Figure 59

CCTTAAAGAA CTGTTCTTTG CAGTGCTCAC ACAGATTGTC TGATGAAAAA CGAGCAGTAA AACCCTCTACA
GGCTTGTAGC TCAGGTGGTT AGAGCGCACC CCTGATAAGG GTGAGGTCGG TGGTTCAAGT CCACTCAGGC
CTACCAAATT TTCCCTGAAT ACTGCGTTGT GAAATAACTC ACATACTGAT GTATGCTTCG TTATTCCACG
CCTTGTCTCA GGAAAAATTA TCGGTAAAGA GGTTCTGACT ACACGATGGG GCTATAGCTC AGCTGGGGAGA
GCGCCTGCTT TGCACGCAGG AGGTCTGCGG TTCGATCCCG CATAGCTCCA CCATATCGTG AGTGTTTACG
AAAAAATACT TCAGAGTGTA CCTGAAAG.GG TTCACTGCGA AGTTTTGCTC TTTAAAAATC TGGATCAAGC
TGAAAATTGA AACACAGAAC AACGAAAGTT GTTCGTGAGT CTCTCAAATT TTCGCAACAC GATGATGAAT
CGTAAGAAAC ATCTTCGGGT TGTGA

(SEQ ID NO 134)

Figure 60

CCTTAAAGAA GCGTACTTTG CAGTGCTCAC ACAGATTGTC TGATGAAAAG TAAATAGCAA GGCGTCTTGC
GAAGCAGACT GATACGTCCC CTTCGTCTAG AGGCCCAGGA CACCGGCCCTT TCACGGCGGT AACAGGGGTT
CGAATCCCCT AGGGGACGCC ACTTGCGCGG TAATGTGTGA AAGCCGTTGCC ATCAGTATCT CAAAACTGAC
TTACGAGTCA CGTTTGAGAT ATTTGCTCTT TAAAAATCTG AAAATTGAAA CACAGAACAA
CGAAAGTTGT TCGTGAGTCT CTCAAATTTT CGCAACACGA TGATGAATCG TAAGAAACAT CTTCGGGTTG
TGA

(SEQ ID NO 135)

Figure 61

CCTTAAAGAA CTGTTCTTTG AAGTGCTCAC ACAGATTGTC TGATGAAAAA CGAGCAGTAA AACCTCTACA
GGCTTGTAGC TCAGGTGGTT AGAGCGCACC CCTGATAAGG GTGAGGTCGG TGGTTCAAGT CCACTCAGGC
CTACCAAATT TTCCCTGAAT ACTGCGTTGT GAAATAACTC ACATACTGAT GTATGCTTCG TTATTCCACG
CCTTGTCTCA GGAAAAATTA TCGGTAAAGA GGTTCTGACT ACACGATGGG GCTATAGCTC AGCTGGGAGA
GCGCCTGCTT TGCACGCAGG AGGTCTGCGG TTCGATCCCG CATAGCTCCA CCATCTCGTG AGTGTTTACG
AAAAAATACT TCAGAGTGTA CCTGAAAGGG TTCACTGCGA AGTTTTGCTC TTTAAAAATC TGGATCAAGC
TGAAAATTGA AACACAGAAC AACGAAAGTT GTTCGTGAGT CTCTCAAATT TTCGCAACAC G

(SEQ ID NO 136)

Figure 62

CCTTAAAGAA GCGTACTTTG AAGTGCTCAC ACAGATTGTC TGATGAAAAG TGAATAGCAA GGCGTCTTGC
GATTGAGACT TCAGTGTCCC CTTCGTCTAG AGGCCCAGGA CACCGCCCTT TCACGGCGGT AACAGGGGTT
CGAATCCCCT AGGGGACGCC AGCGTTCAAA CTGATGAGGT CAAACCTCCA GGGACGCCAC TTGCTGGTTT
GTGAGTGAAA GTCACCTGCC TTAATATCTC AAAACTGACT TACGAGTCAC GTTTGAGATA TTTGCTCTTT
AAAAATCTGG ATCAAGCTGA AAATTGAAAC ACAGAACAAC GAAAGTTGTT CGTGAGTCTC TCAAATTTTC
GCAACACGAT GATGAATCGT AAGAAACATC TTCGGGTTGT GA

(SEQ ID NO 137)

<u>Figure 63</u>

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CCTTAAAGAA ACGGTCTTTG AAGTGCTCAC ACAGATTGTC TGATGAAAAA CGAGCAGTAA AACCTCTACA
GGCTTGTAGC TCAGGTGGTT AGAGCGCACC CCTGATAAGG GTGAGGTCGG TGGTTCAAGT CCACTCAGGC
CTACCAAATT TTCCCTGAAT ACTGCGTTGT GAAATAACTC ACATACTGAT GTATGCTTCG TTATTCCACG
CCTTGTCTCA GGAAAAATTA TCGGTAAAGA GGTTCTGACT ACACGATGGG GCTATAGCTC AGCTGGGGAGA
GCGCCTGCTT TGCACGCAGG AGGTCTGCGG TTCGATCCCG CATAGCTCCA CCATCTCGTG AGTGTTTACG
AAAAAATACT TCAGAGTGTA CCTGAAAGGG TTCACTGCGA AGTTTTGCTC TTTAAAAATC TGGATCAAGC
TGAAAATTGA AACACAGAAC AACGAAAGTT GTTCGTGAGT CTCTCAAATT TTCGCAACAC GATGATGAAT
CGTAAGAAAC ATCTTCGGGT TGTGA
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(SEQ ID NO 138)

<u>Figure 64</u>

CTAAGGATAT ATTCGGAACA TCTTCTTCGG AAGATGCGGA ATAACGTGAC ATATTGTATT CAGTTTTGAA
TGTTTATTTA ACATTCAAAT ATTTTTTGGT TAAAGTGATA TTGCTTTTGA AAATAAAGCA GTATGCGAGC
GCTTGACTAA AAAAAATTGT ACATTGAAAA CTAGATAAGT AAGTAAAATA TAGATTTTAC CAAGCAAAAC
CGAGTGAATA AAGAGTTTTA AATAAGCTTG AATTCATAAG AAATAATCGC TAGTGTTCGA AAGAACACTC
ACAAGATTAA TAACGCGTTT AAATCTTTTT ATAAAAGAAC GTAACTTCAT GTTAACGTTT GACTTATAAA
AATGGTGGAA ACATA

(SEQ ID NO 139)

Figure 65

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CTAAGGATAT ATTCGGAACA TCTTCTTCAG AAGATGCGGA ATAACGTGAC ATATTGTATT CAGTTTTGAA
TGTTTATTTA ACATTCAAAT ATTTTTTGGT TAAAGTGATA TTGCTTATGC GAGCNCTTGA CAATCTATTC
TTTTTAAAGA AAGCGGTTGT CAGACAATGC ATTAAGAAAA ATTAAAGCGG AGTTTACTTT TGTAAATGAG
CATTTGATTT TTTGAAAATA AAGCCAGTATG CGAGCGCTTG ACTAAAAAGA AATTGTACAT TGAAAACTAG
ATAAGTAAGT AAAATATAGA TTTTACCAAG CAAAACCGAG TGAATAAAGA GTTTTAAATA AGCTTGAATT
CATAAGAAAT AATCGCTAGT GTTCGAAAGA ACACTCACAA GATTAATAAC GCGTTTAAAT CTTTTTATAA
AAGAAAAACGT TTAGCAGACA ATGAGTTAAA TTATTTTAAA GCAGAGTTTA CTTATGTAAA TGAGCATTTA
AAATAATGAA AACGAAGCCG TATGTGAGCA TTTGACTTAT AAAAATGGTG GAAACATA
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(SEQ ID NO 140)

Figure 66

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CTAAGGATAT ATTCCGGAACA TCTTCTTCAG AAGATGCGGA ATAACGTGAC ATATTGTATT CAGTTTTGAA
TGTTTATTTA ACATTCAAAT ATTTTTTGGT TAAAGTGATA TTGCTTATGC GAGCGCTTGA CAATCTATTC
TTTTAAAGA AAGCGGTTGT CAGACAATGC ATTAAGAAAA ATTAAAGCGG AGTTTACTTT TGTAAATGAG
CATTTGATTT TTTGAAAATA AAGCAGTATG CGAGCGCTTG ACTAAAANGA AATTGTACAT TGAAAACTAG
ATAAGTAAGT AAAATATAGA TTTTACCAAG CAAAACCGAG TGAATAAAGA GTTTTGAATA AGCTTGAATT
CATAAGAAAT AATCGCTAGT GTTCGAAAGA ACACTCACAA GATTAATAAC GCGTTTAAAT CTTTTTATAA
AAGAACGTAA CTTCATGTTA ACGTTTGACT TATAAAAATG GTGGAAACAT A
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(SEQ ID NO 141)

Figure 67

CTAAGGATAT ATTCGGAACA TCTTCTTCAG AAGATGCGGA ATAACGTGAC ATATTGTATT CAGNTTTGAA
TGTTTATTTA ACATTCAAAA AATGGGCCTA TAGCTCAGCT GGTTAGAGCG CACGCCCTGAT AAGCGGTGAGG
TCGGTGGTTC GAGTCCACTT AGGCCCACCA TTATTTGTAC ATTGAAAACT AGATAAGTAA GTAAAATATA
GATTTTACCA AGCAAAACCG AGTGAATAAA GAGTTTTAAA TAAGCTTGAA TTCATAAGAA ATAATCGCTA
GTGTTCGAAA GAACACTCAC AAGATTAATA ACGGCGTTTAA ATCTTTTTAT AAAAGAACGT AACTTCATGT
TAACGTTTGA CTTATAAAAA TGGTGGAAAC ATA

(SEQ ID NO 142)

Figure 68

CTAAGGATAT ATTCGGAACA TCTTCYTCAG AAGATGCGGA ATAATGTGAC ATATTGTATT CAGTTTTGAA
TGTTTATTTA ACATTCAAAT ATTTTTTGGT TAAAGTGATA TTGCTTATGC GAGCGCTTGA CTAAAAAGAA
ATTGTACATT GAAAACTAGA TAAGTAAGTA AAANTATAGA TTTTACCAAG CAAAACCGAG TGAATAAAGA
GTTTTAAATA AGCTTGAATT CATAAGAAAT AATCGCTAGT GTTCGAAAGA ACACTCACAA GATTAATAAC
GCGTTTAAAT CTTTTTATAA AAGAACGTAA CTTCATGTTA ACGTTTGACT TATAAAAATG GTGGAAACAT
A

(SEQ ID NO 143)

Figure 69

CTAAGGATAT ATTCGGAACA TCTTCTACGA AGATGAGGGA ATAACGTGAC ATATTGTATT CAGTTTTGAA
TGTTTATTAA CATTCATTTG TACATTGAAA ACTAGATAAG TAAGTAAGAT TTTACCAAGC AAAAACCGAGT
GAATAGAGTT TTAAATAAGC TTGAATTCAT AAATAATCGC TAGTGTTCGA AAGACNTCCA CAAGATTAAT
AACTAGTTTT AGCTATTTAT TTTGAATAAC AATTCAAAAT ATGGTGGGAC ATA

(SEQ ID NO 144)

Figure 70

AAGGATAAGG AACTGCACAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTTGCACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAA CGCTGTAGTA
TTAATAAAGA GTTTATGACT GAAAGGTCAA AAAAATAA

(SEQ ID NO 145)

Figure 71

AAGGAAATGG AACACGTTTA TCGTCTTATT TAGTTTTGAG AGGTCTTGTG GGGCCCTTAGC TCAGCTGGGA

GAGCGCCTGC TTNGCACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATCAGGATA CANTCCTACT

AAACTTAAATA CAAGTGAAGT TGAACACGCA ACTCACTTCC TAGGAAAATA GACAATCTTC GCTTGTGTGC

AAGGCACACA TGGTCAGATT CCTAATTTTC TACAGAAGTT TCGCTAAAGC GAGCGTTGCT TAGTATCCTA

TATAAATAGTC CATNGAAAAT TGAATATCTA TATCAAATTC CACGATCTAG AAATAGATTG TGGAAAACGTA

ACAAGAAATT AACCCGNAAA CGCTG

(SEQ ID NO 146)

Figure 72

AAGGATAAGG AACTGCACAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTG GGGCCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTTGCACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAC GCTGTAGTAT
TAAAAGAGTT TATGACTGAA AGGTCAGAAA ATAA

(SEQ ID NO 147)

Figure 73

CTAAGGATAT ATTCGGAACA TCTTCTTACG AAGATGCAGG AATAACATTG ACATATTGTA TTCAGNTGTG
AATGCTCATT GGAGNATTCA TNGCATNATT TGGTNCATTG ACANCTAGAT AAGNAAGTAA AATTTATGAT
TTTACCAAGC AAAACCGAGT GAATTAGAGT TNTNNAACAA GCTTTGATTT CAAAAAGAAA TAATCGCTAG
TGTTCGAAAG AACACTCACA GATTANTAAC ATCTTGGGTT TTCACCCGAC TTGTTCGTNT CGAAAGTCAA
AAAA

(SEQ ID NO 148)

Figure 74

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AAGGATAAGG AACTGCGCAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGGCCTGC TTTGCACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAA CGCTGTAGTA
TTAATAAGAG TTTATGACTG AAAGGTCAAA AAATAA
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(SEQ ID NO 149)

Figure 75

AAGGATAAGG AACTGCGCAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGGCCTGC TTTGCACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAA CGCTGTAGTA
TTAATAAGAG TTTATGACTG AAAGGTCAGA AAAATAA

(SEQ ID NO 150)

Figure 76

AAGGAAAAAGG AACTGCGCAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGGCCTGC TTTGCACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAA CGCTGTAGTA
TTAATAAGAG TTTATGACTG AAAGGTCAGA AAAATAA

(SEQ ID NO 151)

Figure 77

AAGGATAAGG AACTGCGCAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTTGCACGCA GGAGGTCAGC CGCTAGGCTC CATTGGTGAG GGTTCGATCC AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAC GCTGTAGTAT
TAAAAGAGTT TATGACTGAA AGGTCAGAAA ATAA

(SEQ ID NO 152)

Figure 78

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AAGGATAAGG AACTGCGCAT TGGTCTTGTT TAGTCTTGAG AGGTCTTGTG GGGCCTTAGC TCAGCTGGGA
GAGCGCCTGC TTTGCACGCA GGAGGTCAGC GGTTCGATCC CGCTAGGCTC CATTGGTGAG AGATCACCAA
GTAATGCACA TTGAAAATTG AATATCTATA TCAAATAGTA ACAAGAAAAT AAACCGAAAC GCTGTAGTAT
TAAAAGAGTT TATGACTGAA AGGTCAAAAA TAA
```

(SEQ ID NO 153)

Figure 79

TAAGGAAGAT CGAGAATTGG AAAGAGGTCG GATTTATCCG GATGATCCTT CTCCATCTTA TTAGAACATA
GATCGCAGGC CAGTCAGCCT GACGATCGCT TGCAGGCGTG CCGCCTTCGT TTCTCTTTCT TCATTGTTGA
TTGCTCACGG GCCGTACCGC AGCTGACGCT GCTGGCCCTG CGCAGGCGCG GCCCATCAGG GCCGAACGGC
CGGTCGGCCT TGCNAAGCTT CGCTTCGGGG TGGATCTGTG GATCGCGTAG TAGCGTTTGC GTCGGTATCT
GGGCTTGTAG CTCAGTTGGT TAGAGCACAC GCTTGATAAG CGTGGGGTCG GAGGTTCAAG TCCTCCCAGG
CCCACCAAGT TACTTGATGA GGGGCCGTAG CTCAGCTGGG AGAGCACCTG CTTTGCAAGC AGGGGGTCGT
CGGTTCGATC CCGTCCGGCT CCACCATCAT GTTGGTGTTG AGACGGATAT TGGCAATCAA CAAAAGAAAG
AAACAAGTTT GCGGGACTNTT ACGAAAGTCT GCCTGTTCTG TATGAAATCG ATGTAATCGG
ATCAAACTGAA GAGTTGATGT CGCAAGAAGC TTGCTCAAGC CTTGCATAAT GATTGATGTG TTTAACCGCC
ATCACCGATT GTATCTCGAG AAGCTGGTCT TTCTGCTGAT ACTGTTGAAA CGAGCATTTG CAGTCGAATG
GCAACATTCG GCGTCGGCATA ATGCGGGCTTT AAGAGCTGAG TTTTGATGGA TATTGGCAAT GAGAGTGATC
AAGTGTCTTA AGGGCATTGG TGGATGCCTT GGCATGCAC

(SEQ ID NO 154)

Figure 80

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AAGGAGCACC ACGAGAAACA CTCCAATTGG TGGGGTGTAA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GAAGCCGGGT GCACAACAAC AAGCAAGCCA GACACACTAT TGGGTCCTGA GGCAACATCT CTGTTGGTTT
CGGGATGTTG TCCCACCATC TTGGTGGTGG GGTGTGGTGT TTGAGAATTG GATAGTGGTT GCGAGCATCA
ATTGGATGCG CTGCCTTTTG GTGGCGTGTT CTGTTGTGCA ATTTTATTCT TTGGTTTTTG TGTTTAT
```

(SEQ ID NO 157)

Figure 81

AAGGAGCACC ACGAGAAACA CCCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGGCCGGGT GCACAACAAC AGGCAATCGC CGGACACACT ATTGGGCCCT GAGACAACAC TCGGCCGACT
GAGGTCGACG TGGTGTCCCT CCATCTTGGT GGTGGGGTGT GGTGTTTGAG CATTGAATAG TGGTTGCGAG
CATCTAGCCG GATGCGTTCC CCAGTGGTGC GCGTTCGTCA AAAATGTGTA ATTTTTCTTT TGGTTTTTGT
GTTCGT

(SEQ ID NO 158)

Figure 82

AAGGAGCACC ACGAGAAACA CCCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGGCCGGGT GCACAACAAC AGGCAATCGC CGGACACACT ATTGGGCCCT GAGACAACAC TCGGCCGACT
GAGGTCGACG TGGTGTCCCT CCATCTTGGT GGTGGGGTGT GGTGTTTGAG CATTGAATAG TGGTTGCGAG
CATCTAGACG GATGCGTTCC CCAGTGGTGC GCGTTCGTCA AAAATGTGTA ATTTTTCTTT TGGTTTTTGT
GTTCGT

(SEQ ID NO 159)

Figure 83

AAGGAGCACC ACGAGAAACA CCCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGNNCGGGT NNACAACAAC NGCCAATCGC CGGACACACT ATTGGGNCCT GAGACAAACAC TCGGCCGACT
GAGGTCGACG TGGTGTCCCT CCATCTTGGT GGTGGGGTGT GGTGTTTGAG CATTGAATAG TGGTTGCGAG
CATCTAGCCG GATGCGTTCC CCAGTGGGTGC GCGTTCGTCA AAAATGTGTA ATTTTTCTNT TGGTTTTTGT
GTTCGT

(SEQ ID NO 160)

Figure 84

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AAGGAGCACC  ACGAGAAACA  CTCCAATTGG  TGGAGTGTGA  GCCGTGAGGG  GTTCTCGTCT  GTAGTGGACG
AGGGCCGGGT  GCACAACAGC  AGACAATCGC  CAGACACACT  ATTGGGCCCT  GAGACAACAC  TCGGCCCGACT
TTGGTCGACG  TGGTGTCCCT  CCATCTTGGT  GGTGGGGTGT  GGTGTTTGAG  CATTGAATAG  TGGTTGCGAG
CATCTAGACG  GATGCGTTGC  CCTCGGGCCG  CGTGTTCGTC  AAAAATGTGT  AATTTTTTCT  TTTGGTTTTT
GTGTTCGT
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(SEQ ID NO 161)

Figure 85

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AAGGAGCACC ACGAGAAACA CTCCAATTGG TGGAGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
GGAGCCGGGT GCACAACAAC AGGCAATCGC CAGACACACT ATTGGGCCCT GAGACAACAC TCGGCCGGCT
TTGAGTCGAA GTGGTGTCCC TCCATCTTGG TGGTGGGGTG TGGTGTTTGA GCATTGAATA GTGGTTGCGA
GCATCTAGAC GGATGCGTTG CCTTCGGGCC GCGTGTTCGT CAAAAATGTG TAATTTTTTC TTTTGGTTTT
TGTGTTCGT
```

(SEQ ID NO 162)

Figure 86

AGGGAGCACC GNAAACGCAT CCCGCGTGGG GTGTGGGTTC GGCGTGTTGT GGCGTCGGNC CGAGGTGTTG

GGCAGCAGGC AGTAACCNCC GGAACACTGT TGGGTTTTGA GNNAACACCC GTGGTGGTGT TGTGCTCCCC

GTGGTGNCGG GGTGTGGTGT TTGAGTGTTG GATAGTGGTT GCCGAGCATCT GGCAAAGACT GTGGTAAGCG

GTTTTTGTTG ANTGTTTTCT GGTGTTTGT

(SEQ ID NO 163)

Figure 87

AAGGAGCACC ACGAGAAACA CTCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGGNCGGGT GCACAACAAC AGNCAATCGC CAGACACACT ATTGGNCCCT GAGACAACAC TCGGCCGACT
TNGGTTGAAG TGGTGTCCCT CCATCTTGGT GGTGGGGTGT GGTGTTTGAG TATTGGATAG TGGTTGCGAG
CATCTAANTG AACGCGTCGC CGNCAACGGT TACGTGTTCG TTTGTGTAA TTNTTTCTAT TGGTTTTTGT
GTTCGT

(SEQ ID NO 164)

Figure 88

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AAGGAGCACC ACGAGAAACA CTCCAATTGG TGGGGTGTGA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGGGCCGGGT GCACAACAAC AGGCAATCGC CAGACACACT ATTGGNCCCT GAGACAACAC TCGGCCGACT
TTGGTCGAAG TGGTGTCCCC CCATCTTGGT GGTGGGGTGT GGTGTTTGAG TATTGGATAG TGGTTGCGAA
CATCTAAATG AACGCGTTGC CGGCAACGGT TACGTGTTCG TTTTAGTGTA ATTNTTTCTA ATGGTTTTTG
TGTTCGT
```

(SEQ ID NO 165)

Figure 89

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AAGGAGCACC ACGAGACCTG GGCCGGCCCC GCAGATCGCG GGATCAGCTG AGCTTTCAGG CGATTCGTTG
GATGGCCTCG CACCTGTAGT GGGTGGGGGT CTGGTGCACT CAACAAACTT GGCCGTGGGAT GCGGGAAAGC
ATCTGCGGAA AATCATCAGA CACACTATTG GGCTTTGAGA CAACAGGCCC GCAGNCCTGN CCCGTTGGGG
GCAGNGGGTG TGTTGTTGCC TCACTTTGGT GGTGGGGGTG GTGTTTGATT TGTGGATAGT GGTTGCGAGC
ATCTAGCGCG CAGAATGTGT GGTCTCACTC CTTGTGGGTG GGGCCTGGTT TTGTGTGCGA TTGATGTGCA
ATTTCTTTTG AAACTCATTT TTTGGTTTTT GTGTTGT
```

(SEQ ID NO 166)

Figure 90

AAGGAGCACC ACGAAAAACT CCCCAATTGG TGGGGTGTAA GCCGTGAGGG GTTCCCGTCT GTAGTGGACG
GGGGCCGGGT GCGCAACAGC AAGCGAAACG CCGGACACAC TATTGGGTCC TGAGGCAACA CTCGGGGTTTG
TCCCCCTCAG GGATTTTCTG GGTGTTGTCC CACCATCTTG GTGGTGGGGT GTGGTGTTTG AGAATTGGAT
AGTGGTTGCG AGCATCAAAT GGATGCCGTTG CCCCTACGGG TAGCCGTGTTC TTTTGTGCAA TTTTATTCNT
TGGTTTTTGT GTTTGT

(SEQ ID NO 167)

Figure 91

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AAGGAGCACC ACGAGAAGCA CTCCAACTGG TGGGGTGCAA GCCGTGAGGG GTTCTCGTCT GTAGTGGACG
AGAGCCGGGT GCGGACAAAC GAACGAGCCA GACACACTAT TGGGTCCTGA GGCAACACTC GGGCTTGGCC
AGAGCTGTTG TCCCACCATC TTGGTGGTGG GGTGTGGTGT TTGAGAATTG GATAGTGGTT GCGAGCATCA
AATGGATGCG TTGCCCCTAC GGGTGGGCGTG TTCTTTGTG CAATTTTATT CTTTGGTTTT TGTGTTTGT
```

(SEQ ID NO 168)

Figure 92

AAGGAGCACC ACGAAAAACA CCCCAACTGG TGGGGTGTAA GCCGTGAGGG GCTCCCGTCT GTAGTAGACG
GGCGCCGGGT GCGCAACAGC AAGCGAGCCA GACACACTAT TGGGTCCTGA GGCAACACTC GGGCTTGTCT
TGGACTCGTC CAAGAGTGTT GTCCCACCAT CTTGGTGGTG TTTGAGAATT GGATAGTGGT
TGCGAGCATC ANCTGGATGC GTTGCCCCCA GGGGTAGCGT GTTCTTTTGT GCAATTNTAT TCNNTGGTTT
TTGTGTTAGT

(SEQ ID NO 169)

Figure 93

AAGGAGCACC ACGAAAAACA CTCCGCATCC GGTGGGGTGT GAGCCGTGAG GGAGCCCGTG CCTGTAGTGG
GTGTGGGGTTG GGTGCGCGAC AACAAATGGG AAAAATCGCT GGGCACACTA TTGGGCTTTG AGGCAACACC
TGGTTTGTTT TGGGTGGTGT CGCTCCATCT TGGTGGGTGGG GTGTGGGTGTT TGAGTTGTGG ATAGTGGTTG
CGAGCATCTA AGCAAAAGCT GTTGTTTGAC GGTTTTTGTC GAGTGTTGTG TGTGT

(SEQ ID NO 170)

Figure 94

AAGGAGCACC ACGAAAAACA CTCCAATTGG TGGGGTGTAA GCCGTGAGGG GTTCTCATCT GTAGTGGACG
AGAGCCGGGT GCACAACAGC AAATGAATCG CCAGACACAC TGTTGGGTCC TGAGGCAACA CTCAGGCTTG
TCCCATGTTG GGCTTGATCG GGTGCTGTCC CCCCATCTTG GTGGTGGGGT GTGGTGTTTG AGTATTGGAT
AGTGGTTGCG AGCATCTAAA TGGATACGTT GCCAGTAAAT GTGGGCGTATT CATTGAAAAT GTGTAATTTT
CTTCTTTGGT TTTGTGTGT

(SEQ ID NO 171)

Figure 95

AAGGAGCACC ACGAAAAACA CTCCAATTGG TGGGGTGTAA GCCGTGAGGG GTTCTCATCT GTAGTGGACG
AGAGCCGGGT GCACAACAGC AAATGAATCG CCAGACACAC TGTTGGGTCC TGAGGCAACA CTCAGGCTTG
TCCCATGTTG GGCTTGATCG GGTGCTGTCC CCCCATCTTG GTGGTGGGGT GTGGTGTTTG AGTATTGGAT
AGTGGTTGCG AGCATCTAAA TGGATACGTT GCCAGTAATG GTGGGCGTGTT CATTGAAAAT GTGTAATTTT
CTTCTTTGGT TTTGTGTGT

(SEQ ID NO 172)

Figure 96

AAGGAGCACC ACGAAAAACA CTCCAATTGG TGGGGTGTAA GCCGTGAGGG GTTCTCATCT GTAGTGGACG
AGAGCCGGGT GCACAACAGC AAATGAATCG CCAGACACAC TGTTGGGTCC TGAGGCAACA CTCAGGCTTG
TCCCATGTTG GGCTTGATCG GGTGCTGTCC CCCCATCTTG GTGGTGGGGT GTGGTGTTTG AGTATTGGAT
AGTGGTTGCG AGCATCTAAA TGGANACGTT GCCAGTAAATG GTGGCGTGTT CATTGAAAAT GTGTAATTTT
CTTCTTTGGT TTTGTGTGT

(SEQ ID NO 173)

Figure 97

AAGGAGCACC ATTTCTCAGT CGAATGAACT GAGAACATAA AGCCGAGTATC TGTAGTGGAT ACATGCTTGG
TGAATATGTT TTATAAATCC TGTCCACCCC GTGGATAGGT AGTCGGCAAA ACGTCGGACT GTCATAAGAA
TTGAAACGCT GGCACACTGT TGGGTCCTGA GGCAACACAT TGTGTTGTCA CCCTGCTTGG TGGTGGGGTG
TGGTCCTTGA CTTATGGATA GTGGTTGCGA GCATCTAAAC ATAGCCTCGC TCGTTTTCGA GTGAGGCTGG
TTTTTGCAAT TTTATTAGCT

(SEQ ID NO 174)

Figure 98

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CCTAATGATA TTGATTCGCG TGAAGTGCTC ACACAGATTG TCTGATGAAA AAGTAACGAG CAGAAATACC
TTTATAGGCT TGTAGCTCAG GTGGTTAGAG CGCACCCCTG ATAAGGGTGA GGTCGGTGGT TCAAGTCCAC
TCAGGCCTAC CACTTCTCGA AGTGGAAAAG GTACTGCACG TGACTGTATG GGGCTATAGC TCAGCTGGGA
GAGCGCCTGC CTTGCACGCA GGAGGTCAGC GGTTCGATCC CGCTTAGCTC CACCATATAG TCCTGTATTT
CAATACTTCA GAGTGTACTG GCAACAGTAT GCTGCGAAGT ATTTTGCTCT TTAACAATCT GGAACAAGCT
GAAAATTGAA ACATGACAGC TGAAACTTAT CCCTCCGTAG AAGTATTGGG GTAAGGATTA ACCTGTCATA
GAGTCTCTCA AATGTAGCAG CACGAAAGTG GAAACACCTT CGGGTTGTGA
```

(SEQ ID NO 195)

Figure 99

CCTAATGATA TTGATTCGCG TGAAGTGCTC ACACAGATTG TTTGATAGAA ACGTAATGAG CAAAAGCGCT
ACCTGTTGAT GTAATGAGTC ACTGACTCAT GCTGATACGA ACCGATTAAG ACAGTCAGTT TAATCGGATT
TTCGTGTCCC CATCGTCTAG AGGCCTAGGA CACTGCCCTT TCACGGCTGT AACAGGGGTT CGAATCCCCT
TGGGGACGCC ATTCGATAAT GAGTGAAAGA CATTATCACC GGTTCTTGGA ACCGAAAACA TCTTAAAGAT
GACTCTTGCG AGTCGTGTTT AAGATATTGC TCTTTAACAA TCTGGAACAA GCTGAAAATT GAAACATGAC
AGCTGAAACT TATCCCTCCG TAGAAGTATT GGGGTAAGGA TTAACCTGTC ATAGAGTCTC TCAAATGTAG
CAGCACGAAA GTGGAAACAC CTTCGGGTTG TGA

(SEQ ID NO 196)

Figure 100

TAAGGATAAG GAAGAAGCCT GAGAAGGTTT CTGACTAGGT TGGGCAAGCA TTTATATGTA AGAGCAAGCA

TTCTATTTCA TTTGTGTTGT TAAGAGTAGC GCGGTGAGGA CGAGACATAT AGTTTGTGAT CAAGTATGTT

ATTGTAAAGA AATAATCATG GTAACAAGTA TATTTCACGC ATAATAAATAG ACGTTTAAAGA GTATTTGTCT

TTTAGGTGAA GTGCTTGCAT GGATCTATAG AAATTACA

(SEQ ID NO 197)

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Figure 101

TAAGGATAAG GAAACCTGTG AATCTTTTTC CCTTCTTTTG TTCAGTTTTG AGAGGTTCAT CTCTCAAAAC
GTGTTCTTTG AAAACTAGAT AAGAAAAGTT AGTGTAAAAA GACGAAGAGA AACCGTAGGT TTTTCTTCAA
CCAAAACCGA GAATCAAACC CTTTCCGTTT TCATAAGCGA TCGCACGTTT ATGAAAACAC
AACAACACCT TCGTAAGAAG GATGA

(SEQ ID NO 213)

Figure 102

TAAGGATAAG GAAACCTGTG AATCTTTTTC CCTTCTTTTG TTCAGTTTTG AGAGGTCAAT GACGCTCATA
CTGAGTACCA GGTGACACGT TTTTGAGGTG TCTCTTCGTA TGAGGGGCCT ATAGCTCAGC TGGTTAGAGC
GCACGCCTGA TAAGCGTGAG GTCGGTGGTT CGAGTCCACT TAGGCCCACT TTTTTGAATA AACCTTTCTT
TTTTATATGT TAATAAGGGG CCTTAGCTCA CAAAGATAGT TTGTTCTTTG AAAACTAGAT AAGAAAAGTT AGTGTAAAAA
TCGATCCCGC TAGGCTCCAC TTTTCTTCAA CCAAAACCGA GAATCAAACC GAGAAAGAAT CTTTCCGTTT
GACGAAGAGA AACCGTAGGT TTTTCTTCAA CCAAAACCGA AACAACACCT TCGTAAGAAG GATGA

          TCATAAGCCGA TCGCACGTTT ATGAAAACAC AACAACACCT TCGTAAGAAG GATGA

(SEQ ID NO 214)

Figure 103

TAAGGATAAG GAAACCTGTG AATCTTTTTC CCTTCTTTTG TTCAGTTTTG AGAGGTCAAT GACGCTCATA
CTGAGTACCA GGTGACACGT TTTTGAGGTG TCTCTTCGTA TGAGGGGCCT ATAGCTCAGC TGGTTAGAGC
GCACGCCTGA TAAGCGTGAG GTCGGTGGTT CGAGTCCACT TAGGCCCACT TTTTTGAATA AACCTTTCTT
TTTTATATGT TAATAAGGGG CCTTAGCTCA GCTGGGAGAG CGCCTGCTTT GCACGCCAGGA GGTCAGCGGT
TCGATCCCGC TAGGCTCCAC CAAAGATAGT TTGTTCTTTG AAAACTAGAT AAGAAAAGTT AGTGTAAAAA
GACGAAGAGA AACCGTAGGT TTTTCTTCAA CCAAAACCGA GAAAGAATCT TTCCGTTTTC ATAAGGGATC
GCACGTTTAT GAAAACACAA CAACACCTTC GTAAGAAGGA TGA

(SEQ ID NO 215)

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 04201 (AMOCO CORPORATION) 4 March 1993 see page 19, line 12 - line 15	1,7,45
X	MICROBIOLOGY, vol.140, no.5, 4, READING GB pages 1103 - 1108 J.W. VAN DER GIESSEN ET AL. see the whole document	1,7,10, 45
X	MICROBIOLOGY, vol.140, no.1, 4, READING GB pages 123 - 132 Y. JI ET AL see the whole document	1,7,20

-/--

X	Further documents are listed in the continuation of box C.		X	Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 1995	0 5. 1 2 . 9 5

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	De Kok, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 452 596 (N.V.INNOGENETICS S.A.) 23 October 1991 see page 1, line 1 - page 6, line 58; claims 1,2 & WO,A,91 16454 cited in the application ---	1
X	EP,A,0 395 292 (T.G.BARRY ET AL.) 31 October 1990 cited in the application see page 3, line 27 - line 52 see page 4, line 28 - page 5, line 18 see page 8, line 5 - page 9, line 28 ---	1,7,8
X	JOURNAL OF BACTERIOLOGY, vol.175, no.10, 3, BALTIMORE US pages 2818 - 2825 R. FROTHINGHAM ET AL. cited in the application see the whole document ---	1,7,9, 11,18,45
X	JOURNAL OF INFECTIOUS DISEASES, vol.169, no.2, , CHICAGO US pages 305 - 312 R. FROTHINGHAM ET AL. see the whole document ---	1,7-11, 13,19,22
X	JOURNAL OF BACTERIOLOGY, vol.170, no.6, , BALTIMORE US pages 2886 - 2889 Y. SUZUKI ET AL. see the whole document ---	1,7,8
X	JOURNAL OF GENERAL MICROBIOLOGY, vol.138, no.8, , LONDON GB pages 1717 - 1727 K.E. KEMPSELL ET AL. cited in the application see the whole document ---	1,7,8
A	FR,A,2 683 227 (INSTITUT PASTEUR) 7 May 1993 see the whole document ---	1,7,45
A	FR,A,2 651 505 (INSTITUT PASTEUR) 8 March 1991 see page 7, line 24 - line 31 ---	1,7,45
A	METHODS IN MOLECULAR AND CELLULAR BIOLOGY, vol.5, no.1, 4, NEW YORK US pages 3 - 12 T.M. SCHMIDT see the whole document -----	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

   12 inventions. See additional sheet PCT/ISA/210

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   1,7-24,45 and partly 2,3,46-49

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

                             ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/

claims:

1. 1,7-24,45 and partly 2,3,46-49
2. 25-27 and partly 4,46-49
3. 28,29 and partly 46-49
4. 30-32 and partly 5,46-49
5. 33,34 and partly 46-49
6. 35,36,44 and partly 3,5,46-49
7. 37 and partly 5,46-49
8. 6,38 and partly 5,46-49
9. 39-41,43 and partly 4,46-49
10. 42 and partly 46-49
11. 50,52 and partly 5,6,47-49
12. 51 and partly 5,46-49

1. A method to detect and identify one or more Mycobacterium species and subspecies in a sample.

2. A method to detect and identify one or more Mycoplasma strains in a sample.

3. A method to detect and identify one or more Pseudomonas strains in a sample.

4. A method to detect and identify one or more Staphylococcus strains in a sample.

5. A method to detect and identify one or more Acinetobacter strains in a sample.

6. A method to detect and identify one ore more Listeria strains in a sample.

7. A method to detect and identify one or more Brucella strains in a sample.

8. A method to detect and identify one or more Salmonella strains in a sample.

9. A method to detect and identify one or more Chlamydia strains in a sample.

10. A method to detect and identify one or more Streptococcus in a sample.

11. A method to detect and identify one or more Yersinia strains in a sample.

12. A method to detect and identify one or more Brucella species in a sample.

INTERNATIONAL SEARCH REPORT

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		JP-T-	6502312	17-03-94
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